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INTRODUCTION TO
VERTEBRATE EMBRYOLOGY

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Textbook of General Biology.

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INTRODUCTION TO VERTEBRATE EMBRYOLOGY

A TEXTBOOK FOR COLLEGES
AND UNIVERSITIES

BY

WALDO SHUMWAY, PH.D.

Professor of Zoölogy, University of Illinois

THIRD EDITION, REVISED AND ENLARGED
WITH 525 DRAWINGS COMBINED IN 240 FIGURES

NEW YORK
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To
E. S. S. AND F. S. S.

PREFACE TO THIRD EDITION

In preparing this new edition, I have taken the opportunity to recast and rewrite the first half of the text, incorporating such new material, especially in experimental vertebrate embryology, as I have found helpful in my own teaching, correcting errors which had persisted through earlier editions, and particularly revising the terminology to bring it into line with current American usage.

In recent years I have found that a brief preview of the life histories of the vertebrate types makes an admirable introduction to the study of comparative embryology, familiarizing the students with the essential vocabulary and supplying them with a framework for the more detailed study of development which follows. Such a preview is included as Chapter II. The illustrations of this chapter are not labeled in detail as many of them will be found again with full labeling in later chapters.

Since many of the newer textbooks of general zoölogy give excellent brief accounts of cytology and genetics, the section of this book devoted to these subjects now forms a separate chapter (IV) which may be omitted at the teacher's discretion. It has been completely rewritten, in which task I have been greatly assisted by the publication of Sharp's "Cytology," third edition.

It is impossible, in a text designed for the college rather than the medical school, to ignore the new advances in experimental embryology, especially those directly concerned with vertebrate development. From the wealth of material now available in works of reference (see page 177) I have selected such topics as seemed to have a definite pedagogical value in my own experience. It is hardly to be expected that this selection will meet the needs of all teachers, but it is hoped that it will supply at least a point of departure. With this in mind the material has been segregated into a single chapter (VII) and the reference list made a little more extensive than those in other sections of the book. "Embryology and Genetics" by Morgan, and "The Elements of Experimental Embryology" by Huxley and de Beer

have been of material assistance in the organization of this chapter.

Earlier editions employed, to a degree which I now recognize was extreme for undergraduates, embryological terms current in European texts and manuals for advanced students. This made it difficult for the student to carry on collateral reading in other texts. In this revision the number of technical terms employed has been reduced until there are only 220 which are not encountered also in such recent texts as Curtis and Guthrie's "General Zoölogy," second edition, and Adams' "Introduction to the Vertebrates," representing material provided in courses pre-requisite to embryology. A glossary, including cross references to common synonyms, has been added. Freed from the necessity of acquiring a large new vocabulary, the student, it is hoped, will progress more rapidly to a better understanding of the dynamic aspects of development.

The expansion of the text indicated above has made it impossible to extend the treatment of organogeny to an equal degree, and I have been forced to content myself with a general revision of this section and the substitution of new figures wherever better material has been available than heretofore.

In preparing this revision, I have had the able assistance of Dr. Frank B. Adamstone, associated with me in this laboratory since 1928, and Dr. David H. Thompson, who has been good enough to read the new chapter on chromosomes and the genes. Mr. W. F. Hoheisel, my laboratory assistant, classified a great mass of student queries accumulated through the use of a question-box ever since this text first appeared in mimeograph form. His analysis was most revealing as to the topics where students encountered the greatest difficulties, and these have had special attention in the revision. The new drawings, except certain cuts borrowed from other sources and acknowledged in their respective legends, have been prepared by Mrs. Katharine Hill Paul. It is a pleasure to express my thanks to all those fellow teachers who have made me their debtor by suggestions as to how the text might be made more useful in classroom and laboratory.

WALDO SHUMWAY.

January 5, 1935.

PREFACE TO FIRST EDITION

This book is intended to serve as an introduction to the study of Vertebrate Embryology for undergraduate students in colleges and universities. For this study they have been prepared by completing introductory courses in the principles of biology and the anatomy of the vertebrates. It has been the writer's aim to correlate embryological principles as discussed in lecture and classroom with the anatomy of vertebrate embryos as studied in the laboratory, in such a manner as to produce a text which should be both practical and teachable.

After a general introduction to the subject, a large part of the text is devoted to the subject of early embryology, making use of the comparative method which has been found most successful in the experience of many teachers. Especial emphasis has been laid on four forms: *Amphioxus*, because of the beautiful and diagrammatic simplicity with which the early stages may be seriated; the frog, long an object of laboratory study; the chick, always available for laboratory preparation and observation; and man, whenever human material is available. Following this section, which includes the period of germ-layer formation, the embryonic membranes, and the development of body form, a second division of the book deals with the derivation of the separate organs and organ systems from the germ layers. Here, too, the method is essentially comparative. The general plan by which each organ system develops is first sketched in broad outlines, followed by an account of the divergent details in the frog, chick, and man.

The remainder of the book is intended for laboratory use. In the third section is given a succinct account of the anatomy of each of the more commonly studied stages in the development of the frog, chick, and pig, illustrated by figures of whole mounts and sections selected from the splendid collections at the University of Illinois built up by Professor J. S. Kingsley. The writer has followed the sequential or chronological method in this section, as it has been his experience that this method is as suc-

cessful in the laboratory as is the comparative method in the classroom. It is hoped that from the study of the transparent whole mounts, as well as the transverse, sagittal, and frontal sections, the student may be enabled to visualize the anatomy of embryos in three dimensions. The concluding section of the text deals with methods of preparing embryos for study and of instructions in elementary methods of embryological study. In the writer's experience it is much easier for the student to grasp the relationship of serial sections after he has prepared a set of his own.

In view of the fact that this book has been written primarily for the undergraduate, the writer feels that he need offer no apology for the omission of historical reviews, controversial discussions of obscure phenomena, references to original sources, or lists of synonyms. These neither interest nor profit the beginning student. In the concluding division of the introduction may be found a carefully selected list of handbooks, texts, and atlases to which the more ambitious student may be referred, while a list of references for collateral reading follows each chapter. The brevity of the text is intentional. If the student is informed that every word and sentence is an integral part of the story, he will master it in detail rather than attempt to pick out the more salient points, a procedure for which he is hardly prepared as yet. Brief summaries are appended to the chapters in Parts I and II.

To compensate for the brevity of the text, the reader is provided with a profusion of illustrations, prepared by the well-known scientific artist, Mrs. Katharine Hill Paul. To her skill the writer is deeply indebted. Attention may be called to the fact that no abbreviations are employed in the labeling of figures. The beginning student will be apt to study the illustrations more carefully if he is not compelled to search through lengthy legends to interpret them. Professor E. B. Wilson and Professor J. S. Kingsley have kindly allowed the writer to have several figures from their writings redrawn in order that these might conform to the general style of this text. Messrs. W. B. Saunders have generously consented to the reproduction of certain figures, from "Developmental Anatomy" by Professor L. B. Arey, which had been drawn by Mrs. Paul for that text. The writer is

indebted also to Professor S. H. Gage and the Comstock Publishing Company for the use of an electrotype from "The Microscope." The source of all illustrations not original in this text is acknowledged in the legends.

It is a pleasure to record here a debt of gratitude to Professor J. H. McGregor and Professor J. S. Kingsley for their kindness in reading the original manuscript. Professor H. B. Ward has generously placed the resources of the Department of Zoölogy at the writer's disposal during the preparation of this book. Dr. A. R. Cahn has contributed preparations, suggestions, and — most appreciated of all — uncounted hours in the drudgery of proof-reading and indexing. For assistance in these labors the writer is indebted also to Dr. H. W. Hann and Dr. F. B. Adamstone. If this book serves to help the undergraduate through his first course in embryology, it is due, in no small measure, to the many students who have labored through these pages in mimeographed form and pointed out the difficulties they encountered.

WALDO SHUMWAY.

University of Illinois,
Urbana.

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PART I
INTRODUCTION

INTRODUCTION TO VERTEBRATE EMBRYOLOGY

CHAPTER I

THE STUDY OF EMBRYOLOGY

Embryology may be defined as that division of biological science which deals with the development of the individual organism. It is concerned with the orderly series of changes in form and function through which the initial germ of the new individual is transformed into a sexually mature adult. Among vertebrate animals, at least, the germ with which development commences is normally an egg that has been fertilized by a sperm. The sexually mature adult is an individual which has developed to a point where it can produce mature eggs if a female, or sperms if a male. Sometimes the word ontogeny is used as a synonym for embryology, but more often it is defined to include the entire life history of an individual from its origin to its death.

Early embryologists. — The earliest treatise on embryology which has been preserved is that of Aristotle (384–322 B.C.) entitled “*De Generatione Animalium*,” — concerning the generation of animals. This work describes the reproduction and development of many kinds of animals. It contains the first account of the development of the hen’s egg, day by day, so far as it could be seen with the naked eye. Comparing the different types of reproduction, Aristotle placed the mammals first, for, being unable to discover the egg, he thought that their young arose from a mixture of male and female fluids and were “born alive.” Sharks, on the other hand, arose from eggs which were retained within the body of the mother and so were also born alive. Next he placed the type of reproduction shown by reptiles and birds in which the egg is “complete,” that is to say, furnished with albumen and a shell. Lowest among the vertebrates were

the amphibians and bony fish with "incomplete" eggs. His account of development showed great powers of observation, skill in comparison, and imagination in interpretation. Notable among his speculations is one which has been given the name of "epigenesis." From his observations on the development of the hen's egg he concluded that development always proceeds from a simple formless beginning to the complex organization of the adult.

Another famous name in embryology is that of William Harvey (1578–1657). His book (*"Exercitationes de Generatione Animalium"*) is largely based on the development of the chick, which he described in great detail, although he too was limited by the fact that the microscope had not yet come into general use. One of his contributions was a careful study of the development of the deer, which he compared with that of the chick. From purely theoretical considerations he came to the conclusion that mammals also formed eggs, and is responsible for the dictum *"Ex ovo omnia"* — all animals arise from eggs.

After the invention of the microscope, Marcello Malpighi (1628–1694) published an account of the development of the hen's egg (*"De Ovo Incubato"*), illustrated with excellent figures of development from the 24-hour stage of incubation on. His work was responsible for a theory of "preformation" as opposed to Aristotle's "epigenesis." On theoretical grounds, he held that the various parts of the embryo were contained in the egg and became visible as they increased in size. The enthusiasm resulting from the remarkable discoveries made with the newly invented microscope led to many later and wholly imaginative accounts of homunculi — miniature adults — in eggs or sperms, respectively.

Caspar Friedrich Wolff (1733–1794), in a highly theoretical treatise (*"Theoria Generationis"*), attacked the theory of preformation on logical grounds. A more important contribution on the development of the intestine in the chick demonstrated that the tubular intestine arose from the folding of a flat layer in an earlier stage of incubation. This was a direct refutation of the preformationist idea that the intestine was tubular from the beginning.

Comparative embryology. — Karl Ernst von Baer (1792–1876) is known as the "father of modern embryology." He discovered

the egg of mammals in 1827 and published a book on animal development (1828 and 1837) in which he compared in detail the development of different animals. From these he drew four important conclusions, known as von Baer's laws.

" 1. The more general characteristics of any large group of animals appear in the embryo earlier than the more special characteristics.

" 2. After the more general characteristics those that are less general arise and so on until the most special characteristics appear.

" 3. The embryo of any particular kind of animal grows more unlike the forms of other species instead of passing through them.

" 4. The embryo of a higher species may resemble the embryo of a lower species but never resembles the adult form of that species."

From the time of von Baer up to the present the history of embryology has been marked by increasing specialization. Thus there is a comparative embryology of the vertebrates and a comparative embryology of the invertebrates. There are also other divisions of embryology which will be indicated briefly in the following paragraphs.

Cellular embryology. — Soon after the first volume of von Baer's treatise appeared, Schleiden and Schwann (1838, 1839) announced the cell theory, namely that all living things are composed of, and arise from, living units known as cells. This resulted in an intensive study, commencing in the latter part of the nineteenth century, of the germ cells, their origin and fertilization, which led Sutton in 1901 to the chromosomal theory of inheritance. In 1878 Charles Otis Whitman (1842-1910) traced for the first time the detailed history of the cells formed by the dividing egg (in the leech, *Clepsine*), thereby initiating the study of cell lineage. Cellular embryology is a subject which unites embryology with cytology, the study of the cell and its activities.

Genetics and embryology. — In 1866, Gregor Mendel (1822-1884) first carried on successfully experiments in breeding plants to discover the laws by which individual characteristics are inherited from one generation to another. His conclusions, now known as Mendel's laws, will be discussed in a later chapter. His contributions were long unrecognized, but in 1900 they were

rediscovered and in the following year Sutton first suggested that the behavior of the chromosomes afforded a mechanical explanation of these laws. This has led to the theory of the gene, a name proposed for the unit of heredity by Johannsen (1911). This theory in the hands of T. H. Morgan has assumed great importance to the embryologist, for, to quote from Brachet, "Embryology is fundamentally the study of heredity in action."

Phylogeny and embryology. — In 1866, Ernst Haeckel (1834–1919) published a theory which he believed supported Darwin's theory of evolution and which he called the "fundamental biogenetic law." It is more often known as the recapitulation theory. This theory states that ontogeny is a brief and incomplete recapitulation of phylogeny, or that an animal passes through stages in its development comparable to those through which its ancestors passed in their evolution. So far as the vertebrates are concerned, this would mean that a mammalian embryo should pass through stages which are definitely fish-like and later through stages which are essentially reptilian. The fact is that, although there are individual characteristics which at times are reminiscent of fish-like or reptilian ancestors, there is never a time in the development of a mammal when it could be mistaken for a fish or a reptile. There are evidences that the vertebrates do retain in development certain features which also appeared in the development of their ancestors. For example, clefts appear in the pharynx of the embryos of birds and mammals, opening to the exterior just as they do in the embryos of fish. In the adult fish these clefts contain the gills, but this is not true of adult reptiles or birds. It has been found very difficult, if not impossible, to draw up a genealogical tree of the vertebrates based solely on embryological data, and the recapitulation theory is not so widely accepted as in former times.¹

Experimental embryology. — Among Haeckel's contemporary opponents was Wilhelm His (1831–1904), who directed attention to the physiology of the embryo. Denying the theory of recapitulation, he called attention to the mechanical processes by which the various structures of the embryo arise from particular regions of the germ. Later, Wilhelm Roux (1850–1924) put the study of experimental embryology on a firm basis when he pub-

¹ Shumway. 1932. "The Recapitulation Theory," *Quart. Rev. Biol.* 7:93-99.

lished a program for the new science which he called "the mechanics of development." This has led to an intensive attack upon the problems of development from the physico-chemical side which is carried on actively at the present time. Weismann (1834–1913), a leader in theoretical embryology, suggested a theory of chromosomal inheritance which came very close to the mark. Jacques Loeb (1859–1924) discovered a method of inducing development in unfertilized eggs (artificial parthenogenesis) which has led to extensive research on the nature of fertilization.

TABLE

SOME IMPORTANT EVENTS IN THE HISTORY OF EMBRYOLOGY UP TO 1900

Embryology in the classic period

4th century B.C.

Aristotle

Embryology in the Renaissance period

(Before the general use of the microscope)

1651

Harvey

Epigenesis

(After the general use of the microscope)

1672

Malpighi

Preformation

1768

Wolff

Epigenesis

Embryology in modern times

1839

von Baer

Comparative embryology

1839

(Schleiden and Schwann announced cell theory)

1859

(Darwin announced theory of natural selection)

1866

Haeckel

Biogenetic law

1866

(Mendel announced laws of inheritance)

(Microscopic technique being developed)

1874

His

Experimental embryology

1878

Whitman

Cell lineage

1883

Roux

Mechanics of development

1891

Weismann

Theory of the germplasm

1899

Loeb

Artificial parthenogenesis

1900

(Rediscovery of Mendel's laws)

Chemical embryology. — No attempt is made to mention the names of men still alive who have contributed to our knowledge of embryology, for the roll of distinguished zoölogists here and abroad would have to be called. Yet it may be admissible to comment on the recent appearance of a monumental work in three volumes by Joseph Needham, entitled "Chemical Embry-

ology," which seems to chart the course for still a new subsience in embryology.

The value of embryology. — To the student who specializes in zoölogy, embryology has a particular importance because it deals with the origin and development of the adult body. There is a fascination in tracing out the history of the different anatomical structures as they take form, grow, and gradually assume the appearance familiar to us in the mature animal. And in the history of the different organs are found clues to their relationships and functions. Everyone knows, for example, that the adrenal gland secretes a hormone, epinephrin, which, circulating in the blood, rouses the autonomic nervous system to greater activity. But in embryology the student learns that the part of the adrenal gland which secretes epinephrin is derived from those same ganglia which give rise to the autonomic nerves.

He also finds clues to ancestral relationships. Even though the recapitulation theory has been abandoned as an explanation of development, embryologists recognize that there are structures in the body which correspond to similar parts used for the same or different purposes in the bodies of some distant ancestor. The "retention theory" has been proposed by de Beer as an explanation. Thus the vestigial tail of the human embryo arises in the same place and manner as the tails of other vertebrates, and we have no doubt but that some remote prehuman ancestor sported and made use of a tail. The embryo retains this tail, not as a recapitulation of ancestral history, but because it inherits the genes which initiate the development of a tail. So the student of comparative anatomy often turns to embryology hoping to find homologies in the mode of origin and manner of development of the adult organs in which he is interested.

The modern student of embryology is concerned mainly with the dynamics of development. He examines the protoplasmic organization of the egg and the sperm, their genetic constitution, and the nature of the process in which the sperm initiates development in the egg. He traces the history of the different cells into which the egg divides and tries to learn the way in which that differentiation takes place. He is interested in the mechanics of the processes by which these cells arrange themselves into the different germ layers, and how the different organs arise.

To these problems he brings the methods of descriptive embryology: the delicate technique of preparing embryological material, the skilled use of the microscope and its accessories, the interpretation and reconstruction of his prepared material. He also uses the methods of experimental embryology: the alteration of the normal conditions of development; new genetic complexes; altered environmental conditions at different stages of development; the development of individual cells or parts of the embryo in isolation or transplanted into new positions or different hosts.

Embryology is not an easy subject. It requires a high type of visual imagination. The student must bear in mind that he is dealing with living objects, three-dimensional and continually changing in volume, shape, and constitution. Much of his attention must be given to the cells of the embryo, as they multiply, migrate, take on different appearances, and carry on different functions. But he must always remember that the embryo has a life of its own to lead. All the different cells and cell groups in the embryonic body work in harmony if the development of the embryo is normal. He must not lose sight of the embryo-as-a-whole.

The student preparing for medicine has a professional interest in embryology. Teachers of human anatomy have long since agreed that a knowledge of embryological relationships is the best possible preparation for the study of human anatomy. A good working acquaintance with the outlines of human embryology is prerequisite to the study of obstetrics. And the practitioner of medicine must be prepared to answer all sorts of questions about human development.

There are two different ways of approaching the subject of vertebrate embryology, when more than one type of development is to be studied. By the first method the different types of development are taken up one after another, e.g., amphioxus, frog, chick, man. The second method consists of discussing the different topics of embryology in turn and comparing the conditions found in each of the types. In this book, the second, or comparative, method is employed. But before taking up the first topic in comparative embryology, it is helpful to examine, very briefly, the life history of each of the types to be used in the later discussion. This will serve to introduce the main stages

of embryology, and to point out the different conditions under which development takes place.

SUMMARY

The history of embryology has passed through three phases. First came the period of fact-finding or description. The first name associated with this period is that of Aristotle. Before the invention of the microscope, Harvey, and after its invention, Malpighi, made careful studies of the development of the hen's egg.

The second period is that of comparative embryology commencing with von Baer. Comparative embryology has been influenced in the past by Haeckel's theory of recapitulation, which was supposed to support the Darwinian theory. With this period we associate also the subject of cellular embryology growing out of Schleiden and Schwann's cell theory. This subject is now closely linked with genetics, for the gene, or unit of genetics, is located in the nucleus of the cell.

The present period may be called that of experimental embryology, foreshadowed by His and put on a firm basis by Roux.

The study of embryology is of value in understanding the relationships of the parts of the adult body, and the homologies of adult organs in different groups of animals. But its immediate aim is to discover the nature of developmental processes. Its methods are observational and experimental. It is concerned both with the behavior of the cells of the embryo and with the activities of the embryo as a whole.

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CHAPTER II

VERTEBRATE LIFE HISTORIES

It is obvious that in an introductory text it is impossible to describe the development of an animal representing each vertebrate group. There are, however, four vertebrates whose embryology has been studied more intensively than any others. These are the amphioxus, the frog, the chick, and man. But before continuing with a brief account of the life history of these vertebrates it is advisable for the student to recall the list of terms which will be used in the descriptions following.

TABLE 2
SOME EXPRESSIONS COMMON IN DESCRIBING EMBRYOS
(*Synonyms in parentheses*)

Anterior (cephalic, cranial, rostral) — head end.

Posterior (caudal) — tail end.

Dorsal — back side.

Ventral — belly side.

Lateral — either right (dextral) side, or left (sinistral) side.

Mesial (median, medial) — middle.

Proximal — nearer the point of reference.

Distal — further from the point of reference.

Transverse (horizontal) — a plane intersecting the antero-posterior axis at right angles, dividing anterior portion from posterior.

Sagittal — the mesial plane of the body or any plane parallel to it, dividing right portion from left.

Frontal (coronal) — any plane at right angles to both transverse and mesial planes dividing dorsal portion from ventral.

Primordium (anlage, Germ.; *ébauche*, Fr.) — the first recognizable stage in the development of any new part of the embryo.

Invagination — the growth of a surface in (toward the point of reference).

Evagination — the growth of a surface out (away from the point of reference).

A. AMPHIOXUS

The amphioxus (*Branchiostoma lanceolatum*) is not really a vertebrate, for it lacks a skull and vertebral column. But because it has a notochord and other chordate structures it is a relative of the vertebrates, a protochordate. By some it is be-

ture, the blastopore, which later narrows as the gastrula increases in length (Fig. 1I).

The germ layers. — The outer layer of the gastrula is known as the ectoderm; the inner one is called the endoderm. It really includes not only the endoderm proper, which is to become the lining of the digestive tube, but also the middle germ layer or chorda-mesoderm, which now occupies the roof of the gastrocoel.

The roof of the gastrocoel soon, 11 or 12 hours after fertilization, develops three longitudinal grooves. The middle one of these folds is to become the notochord; the others give rise each to a series of pouches or enterocoels (Fig. 1J), from which the mesoderm is formed.

The ectoderm immediately over the roof of the gastrocoel is called the neural plate. About 15 hours after fertilization, ectoderm from the ventral side grows up over the blastopore to cover the neural plate (Fig. 1J).

Hatching. — The embryo escapes from its egg envelopes at this time, if not indeed a little earlier. It is now cylindrical in form, flattened on the dorsal surface, its length about twice its diameter. It appears to be about twice the volume of the original egg, owing to the large digestive cavity arising from the gastrocoel. The blastopore is covered by ectoderm from the ventral side, but this opens to the exterior by means of the anterior neuropore (Fig. 1K).

The larva. — After hatching, the embryo still subsists on the remainder of its yolk until the mouth opens, about the fourth day after fertilization. It is then about 1 mm. in length, very slender, and probably of no greater volume than the original egg (Fig. 1L). So soon as the mouth opens and the embryo is able to ingest food from external sources it is called a larva. By now all the organ systems except those connected with reproduction are functioning. For about three months the larva leads a free-swimming existence, making its way to deeper waters (Fig. 1M).

Metamorphosis. — At the end of three months, roughly speaking, the larva has increased in length to an average of 3.5 mm. It now gives up its free-swimming life to burrow in the sands and slowly assume its adult characteristics (Fig. 1N). The ability to produce mature germ cells is first manifested when the animal is about 200 mm. long.

B. THE FROG

The frog (*Rana pipiens*) is one of the anuran amphibia. It is selected as a type of ichthyopsid (fish and amphibia) or anamniote (developing without an amnion). It has been a favorite object of embryological observations and experiments for centuries, and its development is better known than that of any other vertebrate except the chick.

Spawning. — The breeding season of the frog is in the early spring, soon after the ice is off the ponds. The males, emerging first from hibernation, make their way to the breeding grounds, where they congregate and sing in chorus while awaiting the coming of the females. On arrival, each female is seized by a male who grasps her for long periods (amplexus). In the early morning both individuals discharge their germ cells, so that fertilization is external. The egg, about 1.7 mm. in diameter (Fig. 2A), is surrounded with a layer of albumen which swells rapidly, causing the eggs, in masses of 3500 to 4500 (Wright), to adhere to vegetation or to rest on the bottom in shallow water. The yolk, present in the form of platelets, is concentrated in the lower hemisphere of the egg.

Fertilization. — Fertilization is external, but the close contact of the individuals during amplexus ensures that the sperm enters the egg before the swelling of the egg jelly prevents it. The first polar body is formed before fertilization, the second afterwards.

Cleavage. — The rate of cleavage depends upon the temperature, but the first division (Fig. 2B) may occur from one to two hours after fertilization, earlier at high temperatures, later at low ones. Cleavage divides the egg completely, but the third cleavage is unequal so that the four $\frac{1}{8}$ -blastomeres of the animal hemisphere are markedly smaller than the four of the vegetal hemisphere (Fig. 2C). After the third cleavage, the pattern becomes more irregular.

Blastula. — The presence of the large yolk-laden blastomeres in the vegetal hemisphere results in an eccentrically placed blastocoel. Furthermore, cleavage planes tangential to the surface of the blastula produce a layer of blastomeres several cells in thickness.

Gastrula. — The presence of great amounts of yolk prevents any invagination, and gastrulation takes place by overgrowth

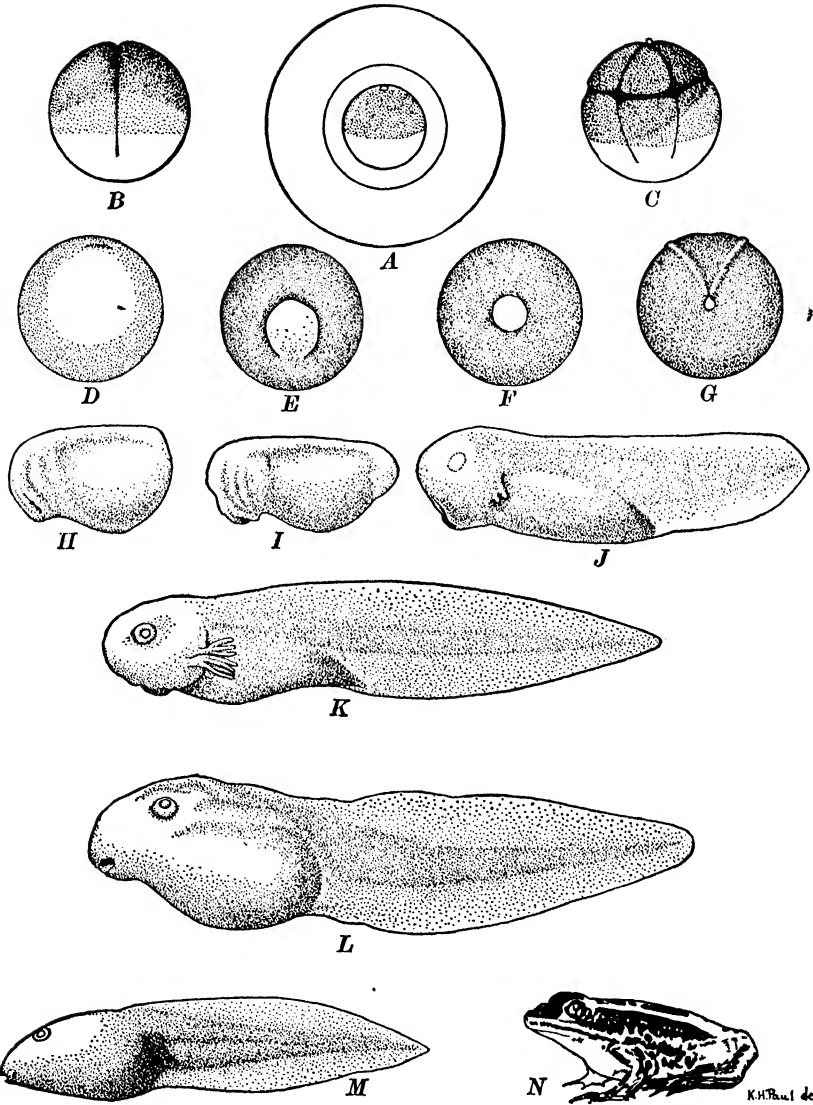


FIG. 2. — Development of the frog. A, fertilized egg, from side, $\times 8$. B, first cleavage, from posterior side, $\times 15$. C, third cleavage, from left side, $\times 15$. D, early gastrula, (dorsal lip stage). E, middle gastrula, (lateral lips). F, late gastrula, (yolk plug stage). G, neural folds. (D–G, from posterior side, semi-diagrammatic, approx. $\times 15$.) H, neural tube closed, 2.4 mm. I, embryo of 3 mm. J, embryo of 6 mm. K, embryo of 9 mm. L, embryo of 11 mm. (H–L, from left side, measured alive, drawn after preservation $\times 10$.) M, full grown tadpole. N, after metamorphosis. (M and N, from left side, $\times 1$, after Wright, 1914.)

instead. Commencing from a shallow groove just below the equator (Fig. 2D), the smaller cells grow down over the larger ones. The down-growing cells form a two-layered fold because the cells at the margin are turned in as the fold grows down. As this overgrowth and tucking-in continues, the fold extends at its two extremities to become crescent-shaped (Fig. 2E). Finally the two ends of the crescent meet to form a circle which rapidly diminishes in circumference until finally only a small plug of the larger yolk-laden cells protrudes (Fig. 2F). The circle is known as the blastopore, and the groove with which it commenced is known as the dorsal lip of the blastopore. A gastrocoel is formed between the large yolk-laden cells and the smaller cells turned in at the lips of the blastopore.

The germ layers. — The cells which were left on the exterior of the gastrula make up the ectoderm, while the inturned cells form an inner layer which includes both the endoderm and the chorda-mesoderm. The roof of the gastrocoel, therefore, is made up of two layers. The lower layer is the endoderm; the upper layer, lying beneath the ectoderm, is the chorda-mesoderm. It separates into three longitudinal strips, of which the middle one becomes the notochord. The others give rise to the mesoderm, which grows down between ectoderm and endoderm. No enterocoels are formed, but the mesoderm breaks up into a series of block-like somites on either side of the notochord.

The neurula. — The neural plate lies over the roof of the gastrocoel. It forms around its margin neural folds (Fig. 2G) which will later grow together to produce the neural tube. At this time the frog embryo is called a neurula. While the neural tube is being formed the embryo increases in length to about 2 mm. This length is attained, ordinarily, on the second day after fertilization, although at room temperature development proceeds more rapidly.

Hatching. — During the first 13 to 20 days the embryo increases rapidly in length and in the development of its organ systems and finally, when it attains the length of 6 mm. (Fig. 2J), it wriggles out of its jelly. At room temperature this may take place within 5 days of fertilization and when the embryo is only 3 mm. in length (Fig. 2I). The embryo as yet has no mouth or external gills but is provided with a sucker by which it attaches

and activities quite unlike those of the adult. This larval period is terminated by a sudden metamorphosis associated with a change to terrestrial conditions.

The egg of the chick is enormous because of the great amount of yolk and the albumen enclosed within the shell. Fertilization is internal and prior to the formation of the albumen and shell. Development is very rapid and accompanied by the development of an amnion or water bath, and an allantois which serves as an extra-embryonic bladder, lung, and albumen sac. These features are correlated with the terrestrial environment of the developing egg. Eggs of this type are termed "cleidoic" (Needham).

The human egg is very small owing to the small amount of contained yolk. Fertilization is internal, and the developing egg soon implants itself in the wall of the uterus where its ten months of development proceed. The early stages of development are passed through very rapidly, and the blastula and gastrula are quite unlike any seen in other classes of vertebrates. An amnion is formed around the developing embryo, and this structure is concerned in the formation of an umbilical cord connecting the embryo to the placenta, a disc-shaped organ of maternal and fetal origin. The placenta serves as an organ of interchange between mother and young up to the time of birth. Development continues long after this event.

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CHAPTER III

THE GERM CELLS

The germ with which the development of the vertebrate commences is the fertilized egg, or zygote. Before discussing the development of the zygote, it is advisable to examine the gametes, egg and sperm, whose union results in its existence. We shall proceed first to the description of the gametes, comparing them with each other and with a generalized cell. Next we shall consider the way in which the germ cells originate and become mature. Thereafter we shall turn to the study of fertilization.

A. THE GAMETES

Vertebrates are characterized by the bisexual method of reproduction, in which there are two distinct sexes: the female, or egg-producing individuals; and the male, or sperm-producing individuals. Among the protochordates (tunicates) we find groups in which the same individual produces both eggs and sperms. Such individuals are called hermaphrodites. This phenomenon is rare among the vertebrates and is not typical of any species.

The two kinds of gametes, eggs and sperms, differ from each other in appearance, size, and structure. These differences will be more apparent after a brief review of cell structure in general.

TABLE 3
STRUCTURE OF THE CELL

- A. Nucleus (composed of karyoplasm).
 - 1. Reticulum (composed of chromatin).
 - 2. Karyolymph (nuclear sap).
 - 3. Nucleolus (plasmosome).
 - 4. Nuclear membrane.
- B. Cytosome (composed of cytoplasm).
 - 1. Hyaloplasm (ground-protoplasm).
 - 2. Centrosomes (centrioles).
 - 3. Mitochondria (chondriosomes).
 - 4. Golgi bodies (dictyosomes).
 - 5. Plastids.
 - 6. Metaplasm (relatively lifeless accumulations).
 - 7. Plasma membrane.
- C. Envelopes or matrix (cell wall).

The cell. — The familiar definition of a cell (Fig. 5) is, “a mass of protoplasm, containing a nucleus, both of which have arisen by the division of the corresponding elements of a preëxisting cell.” Protoplasm in this sense refers to the living substance of the cell, including both the material inside the nucleus and that in the cell body or cytosome. It is customary to use the term karyoplasm (nucleoplasm) for the nuclear protoplasm, and the word cytoplasm for the protoplasm of the cell body. Some writers

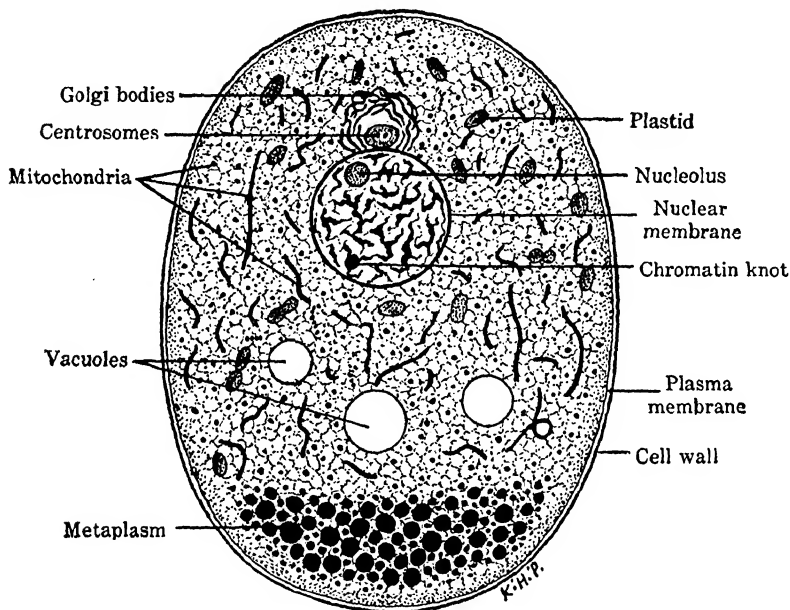


FIG. 5. — Diagram of a composite cell. (After Wilson.)

employ only the words nucleus and cytoplasm to distinguish between nucleus and cell body.

The nucleus. — The cell nucleus is generally a rounded body separated from the cytosome by a delicate nuclear membrane. Within this is a transparent ground substance known as the karyolymph or nuclear sap. But the characteristic substance of the nucleus is its chromatin, a substance staining sharply with basic dyes, and arranged usually in a network of threads called the reticulum (Sharp). Sometimes swellings, chromomeres, are apparent at the nodes of the network. The nucleus usually contains a smaller body known as the nucleolus, a droplet of some

material heavier than the nuclear sap, but staining with acid dyes. Its staining properties alter during cell division.

The nucleus may fragment to form polynuclear cells. It may also divide, often many times, while the cell body remains undivided, resulting in the formation of a syncytium. Sometimes the nucleus may be ejected to leave enucleate cells such as the red blood corpuscles of mammals. But in general every cell has one nucleus.

The type of nucleus here described is known as the vesicular type. There is distinguished also the massive or compact type of nucleus, in which the chromatin forms apparently a solid mass, as in the sperm cell. Then there is a diffuse type, in which the nuclear membrane is absent and the chromatin is scattered through the cell body in granules called chromidia.

The cytosome. — The cytoplasm of the cell body includes an outer delicate semipermeable membrane known as the plasma membrane. This is the surface at which the protoplasm of the cell is in contact with its environment. Within this is the liquid ground substance or hyaloplasm, in which are distributed a number of differentiated bodies. Of these cytoplasmic inclusions the more important seem to be the centrosomes, mitochondria, and Golgi bodies, all of which appear to have the properties of independent growth and division.

The centrosomes (centrioles), small spherical bodies, one or two in number, lie near the nucleus. They seem to be concerned in the process of cell division. In cells with locomotor organs, like the tail of the sperm, the centrosomes are connected with the contractile element of the cell.

The mitochondria (chondriosomes) are small rods, or granules, very numerous and scattered through the cytoplasm. They are dissolved by many common methods of preparing cells for observation, but can be demonstrated in the living cell by a stain called Janus Green B. They are preserved by special chemicals, e.g., osmium tetroxide.

The Golgi bodies (dictyosomes) are sometimes scattered through the cytoplasm but often aggregated into a network, the Golgi apparatus. Some authors deny that there is a real structure of Golgi bodies and speak therefore of the Golgi material or the Golgi zone. Other investigators have sought to identify these

bodies with the plastids, cytoplasmic elements which are found in plant cells. Golgi bodies are hard to identify in living cells but can be demonstrated by special techniques involving the use of osmium tetroxide or silver nitrate. Their function is doubtful, but there is some reason to believe that they are concerned with the elaboration of substances within the cell such as enzymes.

Still another type of inclusion in the cytosome is represented by the plastids. These bodies are found more frequently in plant cells, e.g., chloroplasts, the chlorophyll bodies, which appear to have the capacity of independent growth and division.

Metaplasm is the name given to all those bodies in the cytoplasm which clearly do not possess the properties of independent growth and division. These may be aggregated in vacuoles or distributed in tiny droplets, granules, etc. Among these are such bodies as secretory granules, intermediate stages in the production of cell secretions (enzymes, etc.). Storage granules are end stages in the accumulation of reserve food materials such as yolk, oil, starch, etc. Here also we may include the minute pigment granules. Embryologists sometimes use the term deutoplasm for reserve food materials in the cell.

The cell wall. — In concluding this brief review of cell structures we must recall that the cell may secrete a wall around itself such as the vitelline membrane. In some tissues these cell walls unite to form a matrix such as the intercellular substance of cartilage or bone.

The sperm. — The male germ cell of vertebrates is a very minute flagellate cell ranging in size from 20 microns (crocodile) to more than 2 mm. (*Discoglossus*, an amphibian). The general shape is that of a tadpole with an excessively long tail, but there are sufficient differences among these tiny cells for them to be identified by specialists.

The sperm (spermatozoön) consists of a head and a tail (Fig. 6). The head contains the nucleus, which is compact and stains very deeply with basic dyes. Here also is the acrosome, usually at the apical end, originating from Golgi bodies, possibly connected with the production of some secretion involved in fertilization. The head is surrounded with a delicate plasma membrane.

The tail consists of three divisions: middle-piece, main-piece, and end-piece. The middle-piece contains two centrosomes.

Yolk. — The bulk of the egg is due to the presence of metaplastm in the form of yolk. This substance contains the principal foodstuffs for the developing embryo. Studies on the yolk of the hen's egg indicate that it contains water (50 per cent), proteins, fats, carbohydrates, inorganic salts, vitamins, pigments, and enzymes (Needham).

The yolk is present in the form of spheres, ovoids, or discs, which stain usually with basic dyes. The yolk tends to accumulate in one hemisphere of the egg, forcing the nucleus into the other. Since the yolk is heavier than the other constituents of the egg, the yolk-laden hemisphere is the lower one when the egg is suspended in water. In large-yolked (macrolecithal, megalecithal) eggs, such as those of the frog and chick, the accumulation of the yolk in one region is so marked that they are known as telolecithal eggs. In small-yolked (microlecithal, oligolecithal) eggs, like those of the amphioxus and of man, the yolk is distributed more generally and they are called isolecithal (homolecithal).

Polarity. — Even in isolecithal eggs there is a visible distinction between the two hemispheres of the egg, so that an axis exists from the center of one hemisphere to that of the other. This, known as the polar axis, is the earliest indication of a differentiation in the egg. The two ends of the axis are known as the poles. The polar bodies, referred to in the preceding chapter, are formed at one of these which is known as the animal (apical) pole. It is sometimes called simply the pole. The other is called the vegetal (vegetative, abapical) pole, sometimes the antipole. The nucleus always lies in the polar axis, more or less towards the animal pole. The yolk shows a gradation from the animal towards the vegetal pole. We shall observe in later chapters that the animal pole marks the anterior end of the developing embryo and the vegetal pole marks the posterior end. There is also reason to believe that the polar axis, in addition to being the first expression of symmetry in the egg, marks a gradient of metabolism (Child). By this is meant that metabolic processes are accelerated at the animal pole and progressively retarded towards the vegetal pole.

A considerable body of evidence shows that the animal pole of the egg is the one which was most active in physiological exchange with its environment while still in the ovary. It is the pole of the egg which is attached to the ovary in the amphioxus (Conklin)

and the chick (Conklin). It has been suggested that in the frog the animal pole of the egg is the one lying nearest the arterial blood supply (Bellamy).

Egg envelopes. — The ovum usually possesses, in addition to the plasma membrane, a variety of protective envelopes which are divided into three classes according to the mode of their formation. Primary envelopes are those formed by the egg itself, such as the delicate vitelline membrane. The secondary envelopes are those formed by the follicle cells which immediately sur-

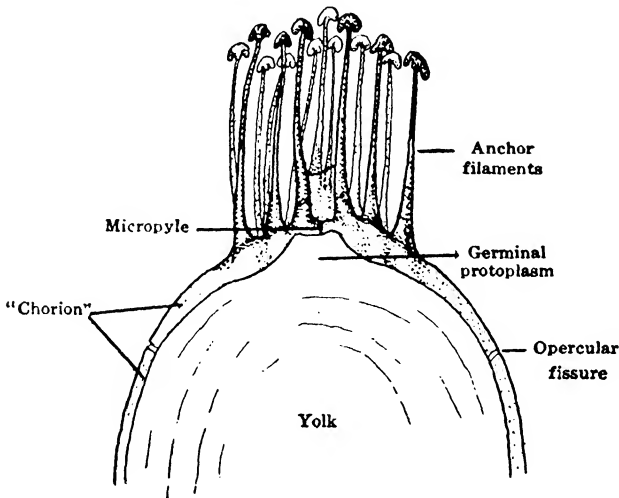


FIG. 9. — Egg of *Myxine*, showing "chorion" and micropyle (after Dean).

round the egg in the ovary. A good example is the so-called "chorion" of one of the cyclostomes, *Myxine* (Fig. 9). It is usually quite difficult to distinguish primary from secondary envelopes, and it is probable that many vitelline membranes are compound in origin. In those vertebrates in which fertilization is external, such as the cyclostomes and bony fish, the primary and secondary envelopes are often perforated by openings called micropyles through which the sperm may have access to the egg. The tertiary envelopes include all those formed by the walls of the oviduct during the passage of the egg. Examples are the egg albumen, shell membranes, and shells of such groups as the reptiles, birds, and the egg-laying mammals; the egg capsules of the elasmobranchs, and the egg jelly of the amphibia and many bony

fish. These envelopes are not formed until after fertilization, except in the case of the egg jelly, and this does not attain its final thickness until after the entrance of the sperm, when it swells by the absorption of water.

THE EGG OF THE AMPHIOXUS. — The eggs (Fig. 10A) are 0.1 mm. in diameter. Before maturation the large nucleus is roughly 0.05 mm. in diameter displaced well towards the animal pole. The cytoplasm consists of a thin outer layer relatively free from

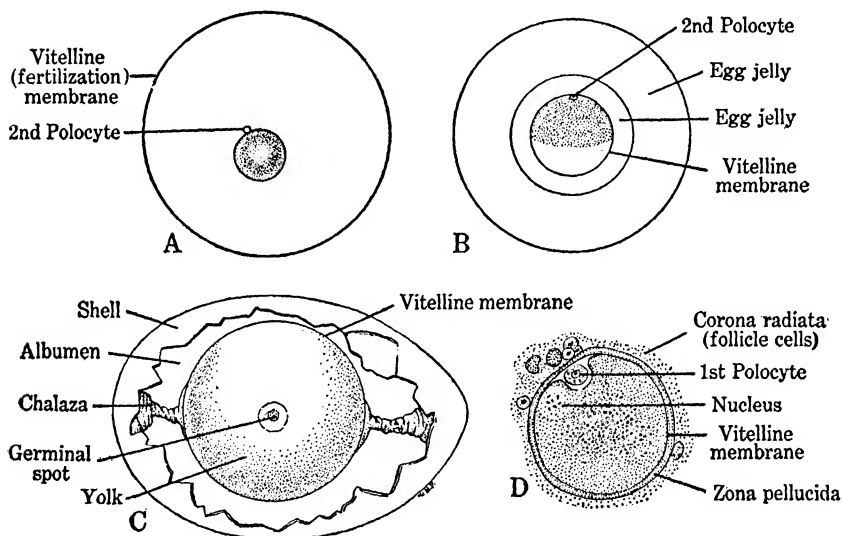


FIG. 10. — Typical eggs. A, amphioxus, approx. $\times 70$ (after Wilson in Willey). B, frog $\times 8$. C, hen $\times \frac{1}{4}$ (after Duval). D, human $\times 250$ (after Allen in Arey).

yolk, and probably containing mitochondria. The rest of the cytoplasm contains yolk. There are no egg envelopes except perhaps a vitelline membrane. The egg is classed as isolecithal.

THE EGG OF THE FROG. — The diameter of the egg (Fig. 10B) is 1.7 mm. (*R. pipiens*, Wright), with a large nucleus before maturation. There is a thin outer layer of cytoplasm, containing granules of pigment in the animal hemisphere. Pigment is also found around the nucleus. The yolk is distributed with fewer and smaller platelets in the animal hemisphere grading down to more and larger platelets in the vegetal hemisphere. There are a vitelline membrane (primary), "chorion" (secondary), and one to three layers of egg jelly (tertiary). The eggs are discharged

in large masses which adhere to each other by means of this jelly. The eggs are classified as telolecithal.

THE EGG OF THE CHICK.—The hen's egg (Fig. 10C) is extremely telolecithal. The cytoplasm, with the nucleus in its center, forms a small germinal disc upon the great mass of yolk.

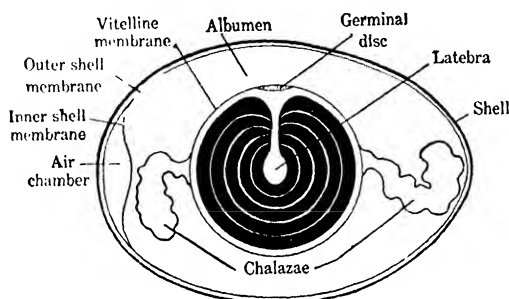


FIG. 11. — Diagram of hen's egg sectioned (after Lillie).

This yolk is arranged in concentric layers of yellow and white material around a central mass of white yolk, called the latebra (Fig. 11). From this latebra a stalk of white yolk (the neck of the latebra) extends upward. The germinal disc rests on this isthmus. The yolk

and germinal disc are surrounded by a delicate vitelline membrane (primary). This in turn is surrounded by the albumen, a viscous tertiary membrane twisted spirally about the egg from left to right, starting from the broad end of the egg. The albumen next to the vitelline membrane is denser than the rest and is prolonged into two spirally twisted cords, the chalazae, one at either end of the egg. The albumen is in turn surrounded by two parchment-like shell membranes, of which the inner one is the thinner. These two are separated at the blunt end of the egg, thus forming the air chamber. The egg shell is a calcareous deposit upon the outer shell membrane. Its color is due to bile pigments of the hen. The germinal disc is about 4 mm. in diameter, the yolk about 40 mm. The size of the egg as a whole varies largely, depending on the amount of albumen deposited around the yolk.

Giant and dwarf eggs are sometimes recorded. In the hen's egg, double- and triple-yolked eggs are known, as well as those which have no yolk at all. A very strange abnormality is known as the "ovum in ovo," where one egg is formed around another. The eggs of birds are either male-producing or female-producing, a statement based solely on the evidence of genetics as no visible differences have been observed.

THE EGG OF MAN. — The human egg (Fig. 10D) is extremely small. The yolk granules are concentrated about the nucleus, which is slightly excentric. It is not positively known whether a vitelline membrane is present. But the egg is enclosed in a thick capsule with radial striations (canals?), the zona pellucida. It is not clear whether this is a primary or secondary envelope. At the time the egg leaves the ovary it is still surrounded by a layer of follicle cells which make up the so-called corona radiata (Fig. 12). The egg may be termed isolecithal. Its diameter is about 0.13 mm.

Eggs and their environment. — Needham has recently pointed out that eggs differ from one another in respect to the physico-chemical constitution of the unfertilized egg, and the possibility

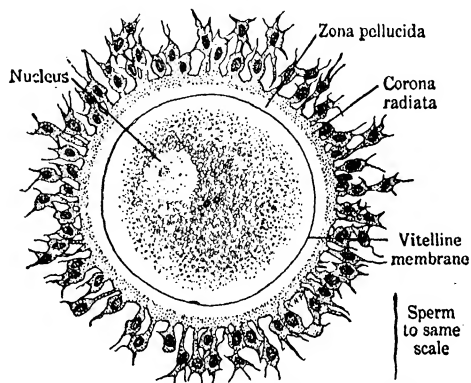


Fig. 12. — Human egg (ovarian) $\times 200$ (after Waldeyer).

of obtaining necessary material from the environment. The marine egg, exemplified by the amphioxus, develops in a medium containing oxygen and inorganic salts. The egg is organized in such a manner as to facilitate the exchange of materials with the environment, and the yolk is small in amount and (to judge from analyses made on marine fish) relatively poor in fats and inorganic salts. Development is rapid up to the hatching stage, but thereafter the larva takes a long time to attain its full size and sexual maturity.

The egg which develops in fresh water, like that of the frog, does not have a medium so rich in salts as the marine egg. It is therefore originally equipped with a larger store of this material. But the aqueous medium still affords facilities for the exchange of carbon dioxide and oxygen and for the disposal of nitrogenous wastes. The jelly with which the frog's egg is provided consists almost entirely of protein and water. Diffusion takes place through it readily, and it affords protection against mechanical

injury and bacterial infection, as well as furnishing a source of nourishment immediately after hatching.

The terrestrial (cleidoic) egg, such as that of the hen, stands easily first in respect to the amount of yolk present. The ratio of fat to protein in the yolk is also the highest. It is obvious that the egg must contain all the material necessary for growth except free oxygen and water, for these are the only substances passing from the atmosphere through the protective envelopes of the egg. Hence, as pointed out by Milnes-Marshall, except in the earliest stages the chick develops more rapidly than the amphioxus and attains its adult form in a much shorter time. The egg albumen also a source of food is a watery solution of protein with some carbohydrates. As we shall see in later chapters, the relative isolation of the embryo in the cleidoic egg is correlated with the development of its extra-embryonic sacs, i.e., the amnion or water bath, and the allantois which serves in the first instance to store nitrogenous wastes.

The uterine egg, typical of the mammals, is characterized by little yolk, for, from a very early period, its nourishment is derived exclusively from the body of the mother. Accordingly there is a precocious separation of a special layer, the trophoblast, concerned with implantation, and later the development of a special organ of interchange, the placenta.

Comparison of the egg and the sperm. — Both gametes are morphologically complete cells. Each has a nucleus and a cytosome containing representatives of the centrosomes, mitochondria, and Golgi bodies. Each has a plasma membrane. Yet neither is capable of independent, continued existence, for physiologically they are unbalanced. The egg is large, inert, and contains a vast store of metaplastm, is protected by egg envelopes, and has lost the power of continued division. The sperm is small, highly motile, contains little cytoplasm and no metaplastm, is devoid of protective membranes, and in itself has lost the power of continued division. We shall now turn to the study of the development of the germ cells and see how the structural differences, at least, arise.

TABLE 4
COMPARISON OF THE VERTEBRATE EGG AND SPERM

<i>Ovum</i>	<i>Cell structures</i>	<i>Sperm</i>
Large amount One, disappears in maturation Diffuse Diffuse Present Vesicular Present Present	Cytoplasm Centrosomes Mitochondria Golgi bodies Plasma membrane Nucleus Nucleolus Nuclear membrane	Small amount Two, retained in maturation Spiral coil Acrosome Present Compact Indistinguishable Present
<i>Other differences</i>		
Large Quiescent None Egg envelopes Spheroid Few to many	Size Movement Motile organs Protection Shape Numbers produced	Small Swims actively Flagellum None Tadpole Very many

B. GAMETOGENESIS

Gametogenesis is the term applied to the history of the gametes — their origin and development (Fig. 13). The special history of the male gametes is called spermatogenesis, that of the female gametes oögenesis.

The origin of the germ cells. — Weismann is responsible for a theory that the germ cells separate completely from all the other cells of the body (soma cells) at a very early stage in development. There is some evidence for this in the embryology of a few invertebrate animals such as *Ascaris*, a parasitic roundworm. In the very first cleavage of the fertilized egg, the two daughter cells show a striking difference, for when one of the daughter cells divides it retains all the chromatin of its nucleus whereas the other gives up a portion of this material to the cytosome. This phenomenon has been called chromatin diminution, and the cell showing this characteristic becomes a soma cell. The other is

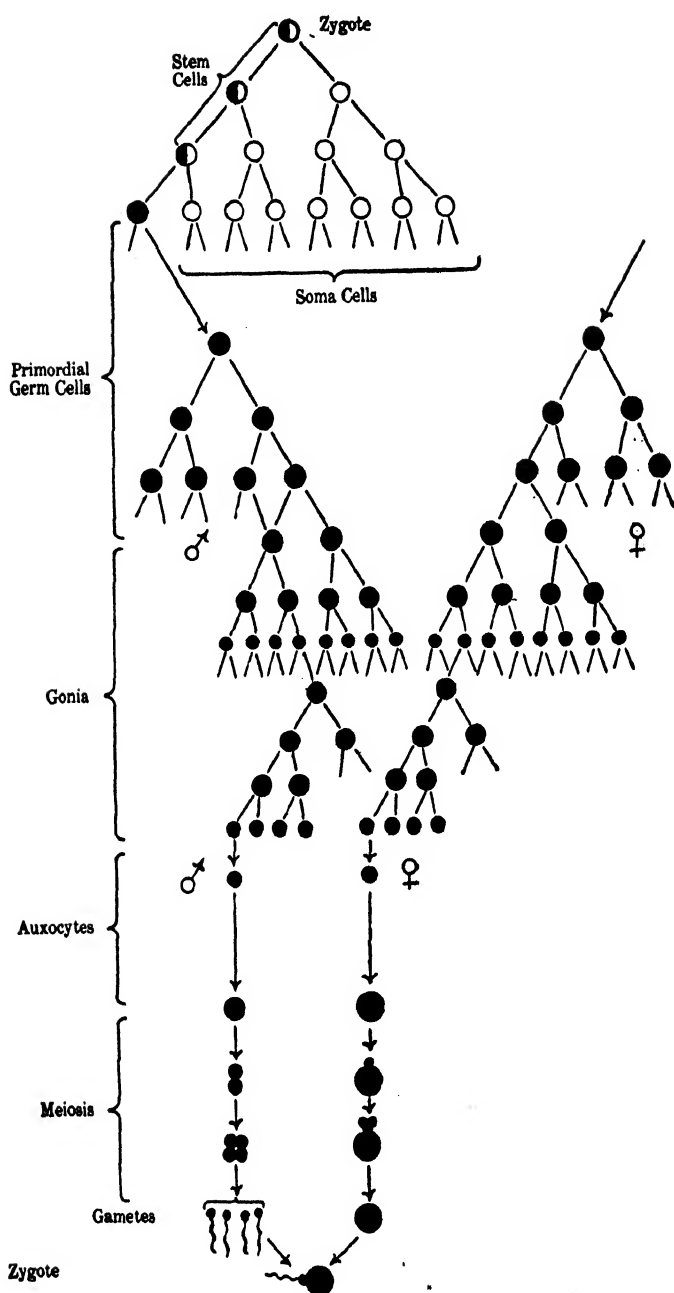


FIG. 13. — Diagram of gametogenesis, male on left, female on right (after Wilson).

known as a stem cell (Fig. 14), and in its division it produces in turn one cell which will be a soma cell and one which will be a germ cell. Eventually a stem cell gives rise to two identical cells, both of which are germ cells. These are known as primor-

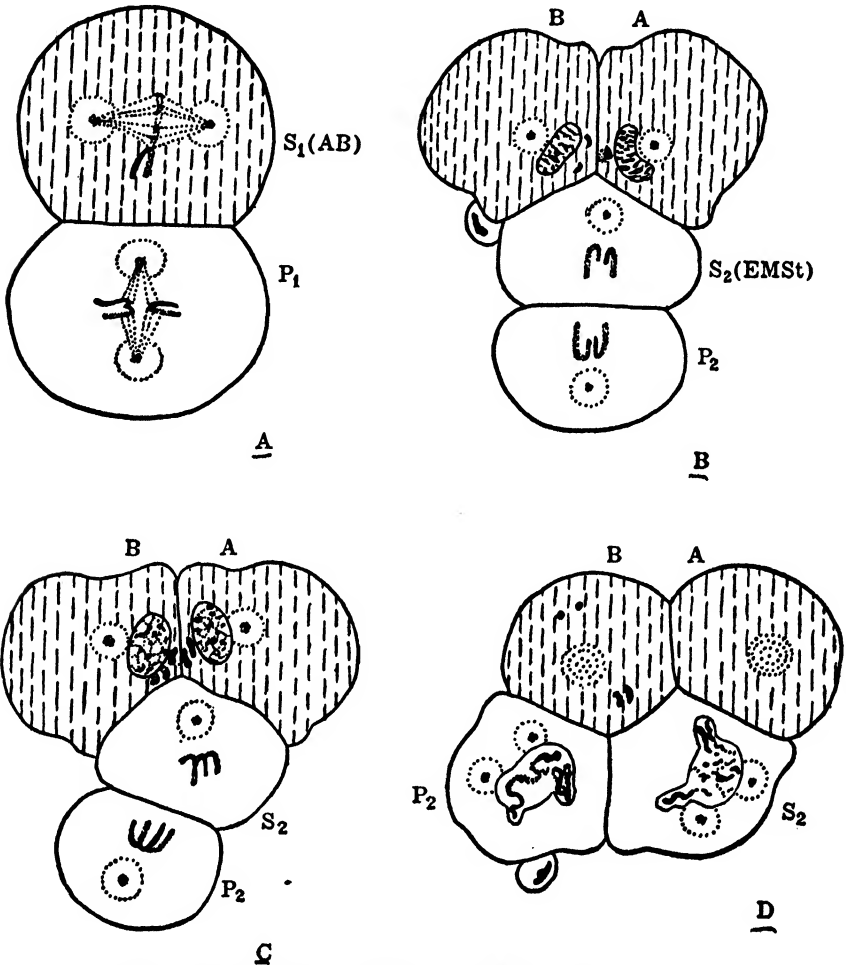


FIG. 14. — Origin of stem cells in *Ascaris*. A, first cleavage. B, C and D, second cleavage. P_1 and P_2 are stem cells. S_1 (which gives rise to A and B) and S_2 are soma cells. (From Richards after Boveri.)

dial germ cells, and, from this time on, they and their descendants produce germ cells only.

This theory of the distinction between germ cells and soma cells has held an important place in the history of biology because

it seemed to deny the possibility of the inheritance of characteristics acquired after fertilization. In other words, the characteristics would be acquired by the soma cells whereas inheritance is transmitted by the germ cells which are entirely distinct. Now that we know that the nuclei of all cells are identical, whether they are germinal or somatic, the theory of the continuity of the germ cells has less theoretical importance.

Primordial germ cells. — In all vertebrates, so far as is known, the germ cells are first recognizable in the lining of the gut at a very early stage of development. These primordial germ cells, as they are called, are distinguishable by their large size, clear cytoplasm, and heavily staining nucleus (Fig. 15). From the gut

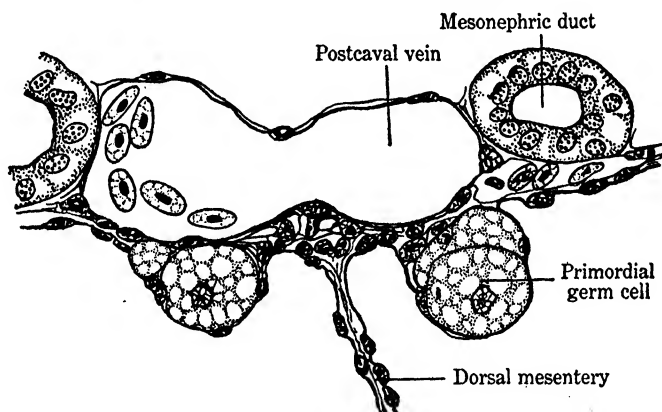


FIG. 15. — Primordial germ cells in the frog (*Rana sylvatica*). Part of transverse section through 10 mm. larva, showing coelomic roof, $\times 375$. (After Witschi, 1929.)

wall they migrate into the mesentery suspending the gut from the roof of the coelom, and thence to the wall of the coelom. Here they multiply rapidly and produce two longitudinal ridges, which are the primordia of the sex glands, or gonads.

The 'gonia. — There are two opinions concerning the fate of the primordial germ cells in vertebrates: one that they give rise to all the later generations of germ cells; the other that they degenerate and the later germ cells arise independently from the tissue of the gonads. In any case, the germ cells which continue to multiply actively in the gonads are known as 'gonia: spermatogonia if they are to give rise to sperm, oögonia if they give rise to eggs.

The 'cytes. — When the individual becomes sexually mature, individual 'gonia undergo a period of growth by means of which they become transformed into 'cytes (auxocytes, meiocytes): spermatocytes if male, oöcytes if female. The 'cyte (Fig. 16) is a large cell with a vesicular nucleus, two centrosomes surrounded by a clear area sometimes known as the sphere substance, which is in turn surrounded by a layer of Golgi bodies, and a cloud of mitochondria.

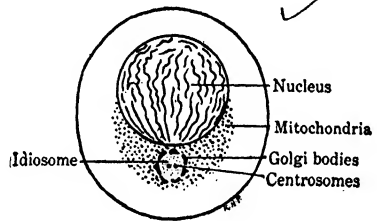


FIG. 16. — Diagram of an early 'cyte (auxocyte). (After Wilson.)

The maturation divisions. — Each 'cyte gives rise to four daughter cells or gones (Sharp) by means of two cell divisions. These divisions are unique because of certain internal phenomena and are known as the maturation divisions. The nature of these divisions will be discussed in more detail in a later chapter (page 64). Meantime we note that the spermatocyte gives rise to four cells of equal size, the spermatids, each of which will be transformed into a sperm. The oöcyte on the contrary gives rise, by the first maturation division, to two cells one of which is very minute, the first polar body (polocyte I). The larger cell undergoes a second unequal division, resulting in the production of a second polar body (polocyte II) and the mature egg or ovum. It will be recalled that among the vertebrates the

TABLE 5
STAGES IN GAMETOGENESIS

<i>Spermatogenesis</i>	<i>General</i>	<i>Oögenesis</i>
Spermatogonia	(Stem cells) Primordial germ cells <i>Period of migration</i> 'Gonia	Oögonia
Spermatocytes	<i>Period of multiplication</i> 'Cytes	Oöcytes
Spermatids <i>Period of metamorphosis</i> Sperms	<i>Period of maturation</i> Gones (Sharp)	Ovum and polocytes (Oötidis)

sperm enters the egg before the production of the second polar body. Sometimes the first polar body also divides so that four cells (oötid) may be produced by the oöcyte. •

Spermatogenesis. — The male 'cyte (primary spermatocyte) is a large cell containing a large vesicular nucleus, more or less excentric. Near the nucleus, and in the center of the thicker layer of cytoplasm surrounding it, are to be seen one or two centrosomes, surrounded by a clear substance known as the sphere substance. This compound body is known as the idiosome, and with it are often associated the Golgi bodies, sometimes so closely connected as to form an investing reticulum or even a shell. Around the idiosome are also grouped the mitochondria forming a cap which sometimes includes the nucleus as well.

The primary spermatocyte divides, giving rise to two secondary spermatocytes, which divide again, often without intermission, each forming two spermatids. The four spermatids thus produced from the primary spermatocyte are later transformed into the sperms.

During the two divisions mentioned above, the chromatin of the spermatocyte nucleus is distributed to the spermatids in such a way that they will differ from each other in respect to the nuclear contents. The details will be discussed later (page 64). The centrosomes divide at each cell division so that each spermatid has a centrosome. The Golgi bodies, each with a small amount of sphere substance, are divided among the four spermatids, in each of which they aggregate to form an idiosome. The mitochondria are divided with almost exact evenness among the spermatids, in each of which they assemble to form a paranucleus (nebenkern). A plasma membrane is present.

In the transformation of the spermatid into the mature sperm (Fig. 17), the nucleus, having previously extruded a large amount of material, condenses into a deeply staining mass which elongates into its final shape. The centrosome divides, and the two centrosomes take up a position which marks the posterior end of the future sperm, one centrosome (proximal) lying against the nucleus, the other (distal) posterior to the first. The paranucleus also takes a posterior position while the idiosome moves around the nucleus to the anterior end. The greater part of the cytoplasm is sloughed off.

Ovulation. — Within the ovary the vertebrate egg is surrounded by nurse cells which make up a nest or follicle (Fig. 19). Within this it enlarges and may undergo its first maturation division. Periodically, varying from once a year in most vertebrates to once a month in the human species, or daily in the domestic fowl, eggs are discharged from the ovary. In numbers this discharge varies from a single egg as in man or the fowl to thousands in the frog or millions in many fish.

The factors bringing about ovulation are diverse. In the frog it has been shown by Rugh¹ that ovulation is brought about by the contraction of a thin muscular layer in each follicle, plus the action of an

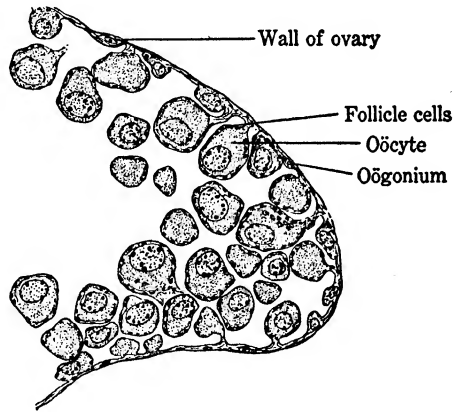


FIG. 19. — Transverse section through part of frog ovary. $\times 95$.

enzyme which digests the outer wall of each follicle and thereby weakens it. In mammals a follicular fluid is secreted about the egg, enlarging the follicle until it protrudes from the surface. Finally the outer wall of the follicle, now very thin, ruptures, owing perhaps to factors similar to those acting on the frog's egg. It has been shown in many vertebrates that ovulation can be induced at any time by means of a hormone secreted by the anterior lobe of the pituitary gland (page 332).

From the ovary the eggs are caught up in the open end of the oviduct, down which they pass to the exterior. In many aquatic forms they are discharged directly. In others they accumulate in an enlarged portion of the oviduct known as the uterus, awaiting discharge from the body; such animals are known as oviparous (amphioxus, frog, chick). In still others the egg remains in the uterus until development has reached an advanced stage; these are the viviparous animals (man, etc.).

Semination. — This term is applied to the discharge of the sperms. These cells remain in the testis (Fig. 20) until mature,

¹ Jour. Exp. Zool. In press.

often attached to nurse cells. When discharged they pass through tubules of the testis which lead directly to a sperm duct. They become motile upon reaching the medium in which fertilization takes place. Enormous numbers are produced at a single discharge (over 200,000,000 in man).

In aquatic animals such as the amphioxus and fish the two sexes congregate together at the breeding season, and eggs and sperms are discharged together. In some cases even aquatic animals have copulatory organs which introduce the sperms into the oviduct, bringing about internal fertilization. In the frog,



FIG. 20. — Section through part of frog testis. $\times 200$.

the males and females unite in pairs (amplexus), thus ensuring that the sperms are discharged simultaneously with the eggs so that fertilization, although external, is regulated. In all terrestrial vertebrates fertilization is internal.

Fertilization. — The actual fertilization of the egg (syngamy) has been observed in the amphioxus and the frog, but our detailed knowledge of the process is obtained from the study of such marine invertebrates as the sea urchin. The essential act in fertilization is the entrance of a single sperm into the egg and the coming together of the two nuclei (pronuclei) (Fig. 21). But this phenomenon is preceded by other events concerned in bringing the sperm to the egg.

Attraction. — One of the factors believed to bring the sperm towards the egg is an attraction (chemotaxis) caused by the emission of some chemical substance by the egg or the female sex

organs. It is known also that sperms swim in a spiral path, and it has been suggested that when they come in contact with a solid object they remain in contact with it (thigmotaxis). If the spiral brings a sperm obliquely towards the egg the contact flattens out the spiral, causing the sperm to remain in contact without penetration. But if the sperm arrives at the egg in a radial direction penetration is facilitated. Lillie has shown that the sea-urchin egg emits a secretion (fertilizin), which brings about a temporary and reversible adhesion of the sperm heads in clusters (agglutination). Fertilizin is produced only after the egg is mature and before it is fertilized.

Penetration. — The sperm bores its way through the egg envelope but then apparently comes to rest against the plasma

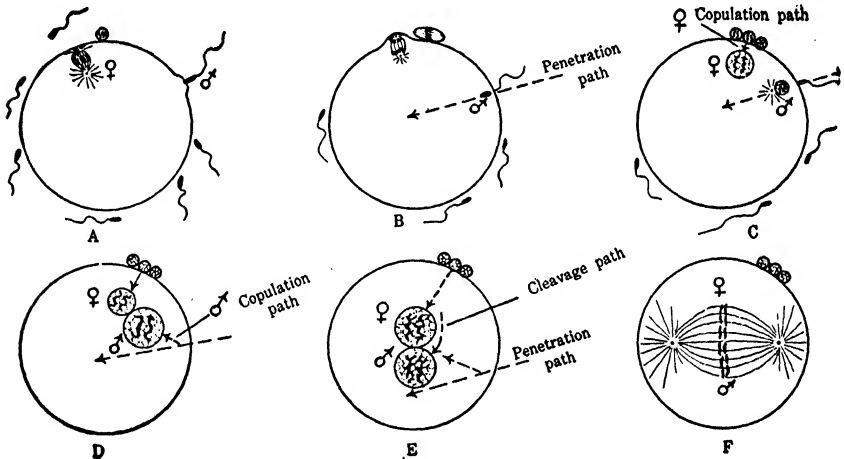


FIG. 21. — Diagram to show fertilization of the egg. A, fertilization cone. B, penetration path. C, female copulation path, and rotation of sperm head. D, male copulation path. E, cleavage path. F, first cleavage.

membrane. Meantime there appears at the surface of the egg a cone or even a long filament of cytoplasm which comes in contact with the sperm head. It then retracts drawing the sperm to the egg and engulfing it (Fig. 21A, B). Thereafter, and commencing at the point where the sperm head was engulfed, a thin layer of surface protoplasm is elevated to form what is known as a fertilization membrane (vitelline membrane?). In older days, when it was thought that the sperm bored its way into the egg, it was believed also that the fertilization membrane acted as a bar to

other sperms. Apparently the elevation of the membrane is due to the secretion of some fluid from the egg, which decreases in diameter at the same time. Okkelberg describes a loss of 14 per cent in the volume of the egg of the brook lamprey. The formation of this membrane with its perivitelline fluid underneath marks the successful fertilization of the egg. For example, the fertilized frog's egg will rotate within this membrane.

The pronuclei. — After the second maturation division, which does not take place in vertebrates until after the entrance of the sperm, the nucleus of the ovum (female pronucleus) is near the periphery at the animal pole, while the nucleus of the sperm (male pronucleus) is at the periphery near the point of penetration. The sperm head rotates 180° so that the male pronucleus now lies distal to the middle-piece containing the centrosome (Fig. 21C). The two pronuclei come together (Fig. 21B, C) by a route which may be analyzed into the following components: (1) the sperm penetration path, which is usually the radius of the egg at which the sperm entered; (2) the sperm copulation path, which is directed towards the point at which the pronuclei will meet and is often at a considerable angle to the penetration path; (3) the egg copulation path, along which the female pronucleus moves towards the meeting point; and (4) the cleavage path (Fig. 21E), along which the two pronuclei move to their final position on the egg axis, often slightly nearer the animal pole. The two pronuclei may unite to form a common reticulum, or they may remain close together contributing independently to the first division of the zygote (Fig. 21F). See also page 156.

The centrosome of the egg disappears after the second maturation division. The centrosome of the zygote, therefore, is either the centrosome of the sperm or, as it is believed in some cases, a new one developed in the egg cytoplasm near the engulfed sperm head.

The mitochondrial material of the sperm fragments and is distributed throughout the cytoplasm of the zygote. The later history of the acrosome has not been followed.

There is much divergence among different kinds of animals with respect to those parts of the sperm which are actually engulfed in the egg. In some, it is the entire sperm; in others, only the sperm head.

Presumptive organ regions. — In many different kinds of eggs, the student of cellular embryology has been able to recognize different regions by differences in the cytoplasm, such as the presence or absence of pigment, mitochondria, yolk, etc., and to trace the distributions of these materials into the different daughter cells as cleavage takes place. These presumptive organ regions, as they may be called, are usually more easily demonstrated after fertilization. For example, before fertilization the living egg of the tunicate *Styela* (*Cynthia*), according to Conklin (1905), has orange pigment granules uniformly distributed in its outer layer of cytoplasm. During fertilization, following an intricate series of stream movements, the orange pigment (Fig. 22) is concentrated in a crescentic area at what will later be the posterior surface. Immediately above this is a similar area of clear protoplasm. On the opposite side of the egg at what will become the anterior surface is a gray crescent. Below these crescents the vegetal hemisphere is marked by the presence of gray yolk. In later

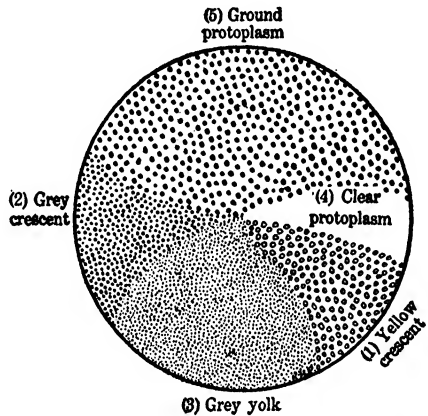


FIG. 22. — Presumptive organ regions (organ-forming substances) in egg of *Styela* after fertilization, viewed from left side, approx. $\times 250$. (After Conklin.)

cleavage, the yellow crescent will be distributed to the cells which form the mesoderm, the gray crescent to the cells which form the notochord and neural plate, the gray yolk to the cells of the endoderm, and the remainder of the egg goes to the cells of the epidermis. The materials contained in the different presumptive organ regions are frequently called organ-forming substances. The regions themselves are called presumptive endoderm, presumptive mesoderm, etc.

Parthenogenesis. — This term is applied to the development of a new individual from an unfertilized egg. It does not occur naturally among the vertebrates but may be illustrated by the honey bee, in which the unfertilized egg develops into a male

(drone) and the fertilized egg becomes a female (either queen or worker according to the type of food supplied).

Artificial parthenogenesis has been produced in the frog's egg by slight punctures with a finely pointed glass needle. Most of the parthenogenetic eggs do not go far in development, but Loeb was able to raise, out of many thousands of treated eggs, a few adult frogs (15 males, 3 females, 2 doubtful).

FERTILIZATION OF THE AMPHIOXUS EGG. — In the case of the amphioxus, fertilization is external. The males and females leave the sands to swarm in the shallow waters during late afternoons of spring and summer. Eggs and sperms are discharged, from the segmental gonads in which they develop, into the cavity of the atrium, and escape to the exterior through the atriopore. The first polocyte is given off before fertilization. Immediately after fertilization the vitelline, now the fertilization, membrane

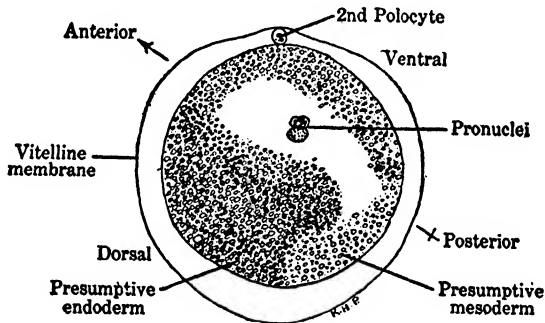


FIG. 23. — Presumptive organ regions in egg of the amphioxus, one hour after fertilization. Sagittal section, approx. $\times 220$. (After Conklin 1932.)

expands greatly, leaving the egg in a large perivitelline space (Figs. 23 and 10A). The second polocyte given off after the formation of the fertilization membrane remains attached to the egg while the first is usually lost to view.

The fertilized egg of the amphioxus (Conklin 1932) shows in sections (Fig. 23) a crescent of more deeply staining protoplasm on the side of the egg which will give rise to the posterior part of the body. This crescent will form the cells of the mesoderm (compare the orange crescent of *Styela*). Opposite this is a less clearly defined crescentic area from which the cells of the notochord and neural plate will be formed (compare the gray crescent

of *Styela* and of the frog). The material of the vegetal hemisphere, bounded above by these crescents, will form the endoderm; the material of the animal hemisphere above the crescents will form the epidermis.

FERTILIZATION OF THE FROG'S EGG. — The fertilization of the frog's egg is external, but the sperm are brought into close proximity to the eggs during the sexual embrace or amplexus. During the breeding season the males embrace the females with the fore-legs, at which time the germ cells of each are extruded. The sperms make their way through the egg jelly before this envelope

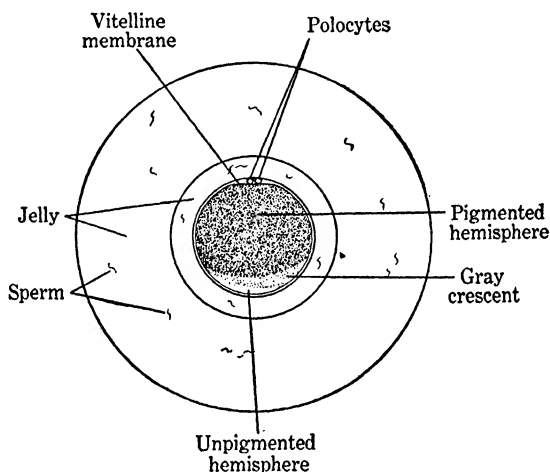


FIG. 24. — Diagram of frog's egg after fertilization to show gray crescent. The line immediately external to the vitelline membrane and polocytes represents the "chorion." $\times 10$.

has attained its final thickness. The entire sperm enters the egg usually within 40° of the apical pole. The vitelline membrane is thrown off as the fertilization membrane, leaving a perivitelline space within which the egg may rotate. The second maturation division then occurs, followed by the conjugation of the pronuclei. The penetration and sperm copulation paths are marked by a trail of pigment dragged in with the sperm head. A single sperm enters the egg. Immediately upon fertilization the cortical cytoplasm of the egg rushes towards the point of penetration, carrying with it the black pigment (melanin) of the animal hemisphere. Upon the side of the egg opposite the point of penetration there appears a crescent-shaped area in which the pigment is less dense

and which is therefore known as the gray crescent (Fig. 24). The region gives rise to the notochord and neural plate.

FERTILIZATION OF THE HEN'S EGG. — In the fowl, fertilization is internal. The sperms, introduced into the cloaca of the female during copulation, make their way to the upper end of the oviduct, where fertilization takes place. Five or six sperms enter the germinal disc, where they remain inactive until after the second maturation division. One of them then moves inward until it comes in contact with the female pronucleus, which has itself moved downward from the surface of the germinal disc. The supernumerary sperms move outward to the border of the disc, where, after a few divisions, they degenerate. The fertilized egg moves slowly down the oviduct while the tertiary envelopes are forming about it.

FERTILIZATION OF THE HUMAN EGG. — Fertilization is internal and occurs at the upper end of the oviduct (Fallopian tube). It

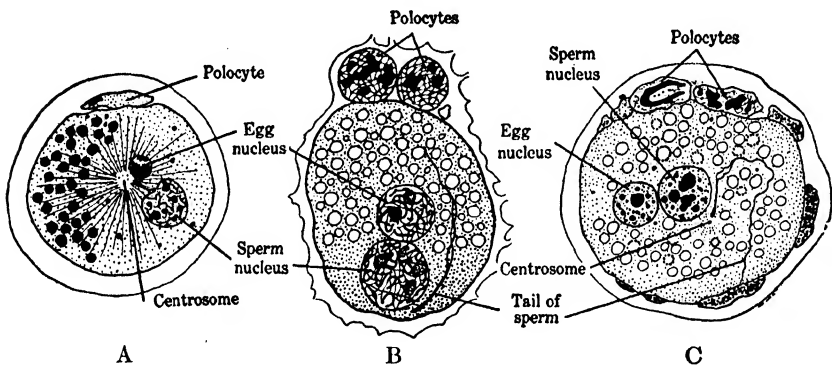


FIG. 25. — Fertilization of the guinea pig egg. Three stages following that shown in Fig. 20A. (After Lams.)

is probable that a single sperm enters the egg after the first maturation division. Further details are lacking, as no direct observations have been recorded. Figure 25 illustrates fertilization in the egg of the guinea pig.

SUMMARY

The gametes are atypical cells, the egg and sperm differing both from each other and from a composite cell. The egg most resembles a composite cell, from which it differs in the absence of a

centrosome after it has become mature. It is large, quiescent, and protected by envelopes. The sperm, almost devoid of cytoplasm, is small, active, and naked.

The gametes are derived from primordial germ cells which are first recognizable in the wall of the gut. Thence they migrate into the roof of the coelom where they multiply rapidly giving rise to the gonad. In the gonad multiplication continues until, when the individual is attaining maturity, some of the 'gonia enlarge to become 'cytes, each of which will undergo two meiotic divisions. The spermatocyte gives rise to four spermatids each of which will be transformed into a sperm. The oöcyte, on the contrary, gives rise to an ovum and two or three polocytes.

The zygote, or fertilized egg, arises from the union of an egg and a sperm. This union is preceded by the discharge of eggs from the ovary (ovulation) and sperms from the testis (semination). The sperm, attracted to the egg, enters it due to the mutual action of the two gametes, and the nuclei of the two gametes come together, each to contribute to the first division of the fertilized egg.

After fertilization, and sometimes even before, it can be seen that the egg has a definite organization, manifest in its polarity (seen even in ovarian eggs) and, in especially favorable material, evident in presumptive organ regions.

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CHAPTER IV

THE CHROMOSOMES AND THE GENES

The germ cells are really cells detached from the bodies of the parents. When they unite in fertilization they bring together material from both parents. Herein lies the explanation of the inheritance of parental characteristics, of the fact that the fertilized egg develops in a way characteristic of the species and the fact that individuals differ from one another. In the following paragraphs we shall review the theory that the individual units of heredity are the genes, borne in the chromosomes, distributed in the maturation divisions, and brought together in fertilization.

A. THE CHROMOSOMES

It will be necessary first to describe the chromosomes as they behave in ordinary (somatic) cell division, then to point out the peculiar features of the maturation (meiotic) divisions and of fertilization, and finally to indicate how this behavior of the chromosomes fits the known laws of heredity.

The chromosomes in mitosis. — The division of most cells is accompanied by the formation and longitudinal division of threads of chromatin, called chromosomes, in the nucleus. This type of cell division is known as mitosis (Fig. 26). Some cells, however, divide without the formation of chromosomes (amitosis), and the daughter cells are thereafter incapable of mitotic division. For the sake of convenience we may use the terms karyokinesis for the division of the nucleus in mitosis and cytokinesis for the division of the cytosome.

Karyokinesis. — Before cell division the metabolic ("resting") nucleus is a reticulum of chromatin lying in the fluid karyolymph with a nucleolus, the whole surrounded with a nuclear membrane (Fig. 26A). In mitosis we distinguish four stages, prophase, metaphase, anaphase, and telophase.

In the prophase the reticulum separates into its constituent threads, chromonemata, by the breaking down of the smaller

threads connecting them. Very early it can be seen that these threads are double or split longitudinally (Fig. 26B). Soon thereafter a matrix is visible about the two chromonemata. This compound structure, consisting of the two chromonemata

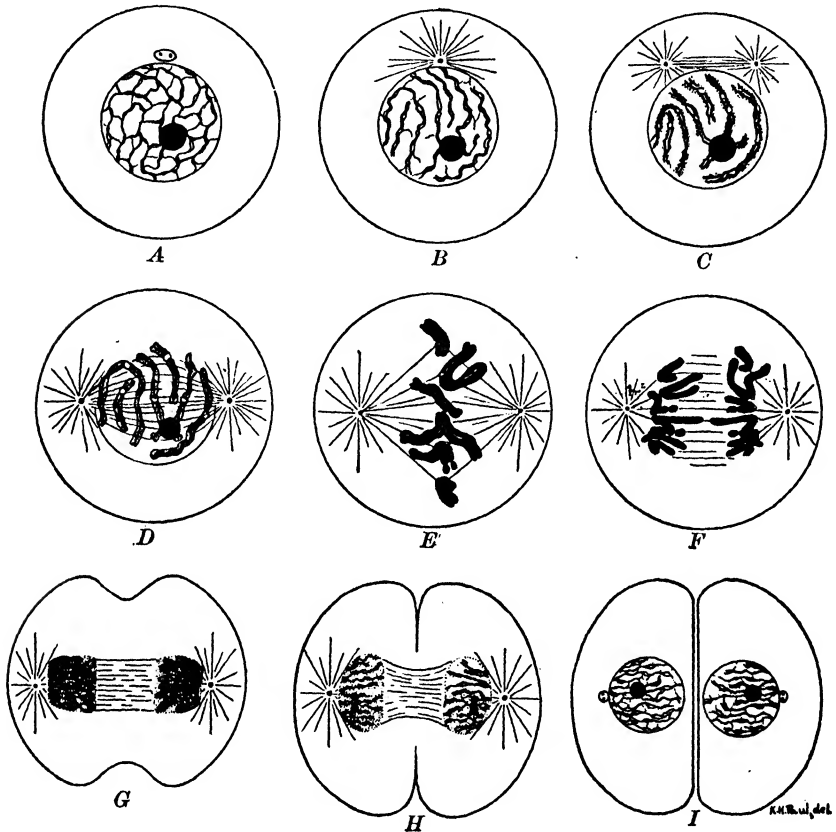


FIG. 26. — Diagrams of somatic mitosis. A, metabolic ("resting") stage. B, early prophase showing chromonemata and attachment points. C, middle prophase, matrix appearing. D, late prophase, chromonemata obscured. E, metaphase. F, anaphase. G, early telophase, matrix disappearing. H, middle telophase, nuclear membrane forming. I, late telophase, reticulum developing. (Based on a diagram by Sharp.)

and the surrounding matrix, is a chromosome (Fig. 26C). The number of chromosomes so formed is the same in every cell of every individual belonging to any particular species. (This statement is subject to exceptions. See pages 69 ff.) Towards the end of the prophase the chromonemata are usually invisible.

Finally the nuclear membrane disappears, and the karyolymph assumes the form of a double cone or spindle (Fig. 26D).

In the metaphase (Fig. 26E), the chromosomes line up in an equatorial plane through the spindle. Each has a definite attachment region lying in the equatorial plane even though the ends of the chromosomes may lie outside of the plane.

In the anaphase (Fig. 26F), the chromosomes separate into two longitudinal portions each containing one of the original chromonemata with surrounding matrix. Preceded always by its attachment region each daughter chromosome moves towards a pole of the spindle. Carothers (1934) describes the growth of a fiber from the attachment region of each daughter chromosome to the nearest pole of the spindle. Eventually two equivalent sets of chromosomes are formed, one in the vicinity of either pole, each set containing a daughter chromosome from each of the original chromosomes formed in the prophase.

In the telophase (Fig. 26G, H, I), each set of chromosomes assumes the metabolic condition. The matrix loses its staining capacity and the chromonemata reappear, often already split longitudinally. The nuclear membrane is formed about each group, the chromonemata are united by tiny cross-strands, the nucleolus reappears, and the nucleus is seen to be filled with karyolymph. The cell now contains two daughter nuclei each identical with the other and with the parent nucleus.

Cytokinesis. — Other striking events are taking place in the cytosome during mitosis. During the prophase the centrosome, if not already divided, separates into two daughter centrosomes which move apart. About each of them is a spherical mass of protoplasm, often containing radial striations, known as the aster. Between them is a central spindle apparently containing fibers. Cytologists distinguish three types of fibers: (1) primary or continuous fibers extending from centrosome to centrosome, (2) half spindle components extending from chromosome to centrosome, and (3) interzonal connections extending between the separating daughter chromosomes (Schrader). The centrosomes reach the opposite sides of the nucleus just as the nuclear membrane disappears. The karyolymph apparently unites with the material between the two centrosomes to form the mitotic spindle along which the chromosomes move in the anaphase. In the telophase,

asters and spindle disappear and the centrosomes alone remain in the positions they occupied at the poles of the mitotic spindle. Sometimes they divide in anticipation of the next mitosis.

The mitochondria usually divide en masse (Fig. 27A). This division of the mitochondria is approximately an equal one, and there is some reason to believe that the individual mitochondria divide during mitosis or just prior to it.

The Golgi bodies, even when aggregated into a Golgi apparatus, separate during mitosis and are segregated into the daughter cells, usually associating themselves with the two centrosomes (Fig. 27B). It is uncertain whether each Golgi body divides individ-

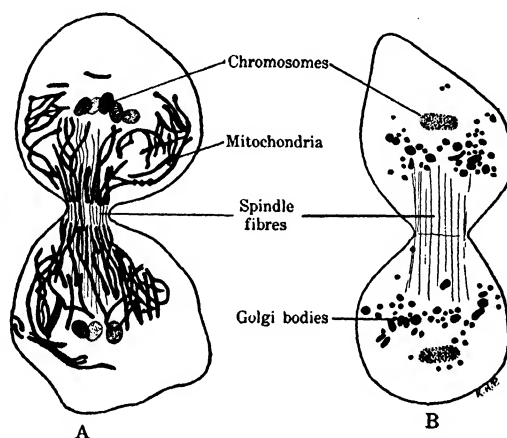


FIG. 27. — The mitochondria and Golgi bodies in mitosis. A, mitochondria. B, Golgi bodies. (After Bowen.)

ually at mitosis, but some evidence has been brought forward to support this contention.

In animal cells the cytosome as a whole divides by constriction. In this process the cell elongates in the direction of the spindle during the anaphase and telophase. Following the reconstruction of the daughter nuclei in the telophase, a furrow appears at the periphery of the cell, around the equatorial belt, and at right angles to the axis of elongation. This furrow advances towards the center of the cell until the cell is completely divided.

Distribution of the chromosomes. — Each daughter cell has approximately half of the cytoplasm proper, half of the mitochondria and Golgi bodies, a centrosome derived from that of the

parent cell, and a nucleus built up from a set of chromosomes, each of which was produced by the division of a chromosome in the parent cell. It is apparent from the foregoing account that the key to the complexities of mitosis is the division of the chromosomes. The achromatic figure is the framework upon which this division takes place. The division of the mitochondria and Golgi bodies is still too little understood. But the chromosomes, appearing in the prophase, halved with such accuracy in metaphase and anaphase, and disappearing again in the telophase, are characterized by a constancy in number, an individuality evinced in form and behavior, and a persistence from generation to generation. In some favorable material it has even been possible to demonstrate that the chromonemata arise in the prophase exactly as they merged into a reticulum in the previous telophase. From the statements above, it is not unreasonable to draw the conclusion that the chromosomes are directly concerned with inheritance in cell reproduction.

The chromosomes in meiosis. — During the two maturation divisions by which the gametes are formed, the number of chromosomes is reduced to one-half the number characteristic of the species. Since in the ordinary somatic mitosis the number of chromosomes given to each daughter cell is exactly the same as that of the parent, it is evident that we are dealing with a peculiar type of mitosis (Fig. 28). The name meiosis is frequently applied to the maturation divisions.

First meiotic division. — The essential feature in which the first meiotic division differs from the ordinary (somatic) mitosis is that during the prophase the chromosomes unite in pairs (Fig. 29, 2). This is synapsis and occurs only in the first meiotic division. Since each of the chromosomes always divides during the prophase also (Fig. 29, 4), the net result is that at the end of the prophase there are only half the number of chromosomes seen in somatic mitosis, but each of these consists of four parts (chromatids) instead of two (Fig. 29, 5). These compound bodies consisting of four chromatids are called tetrads (Fig. 29, 6). The quadripartite nature of the tetrad may be expressed by the formula $\frac{A : a}{A : a}$, where A represents one of the synaptic mates and a the other.

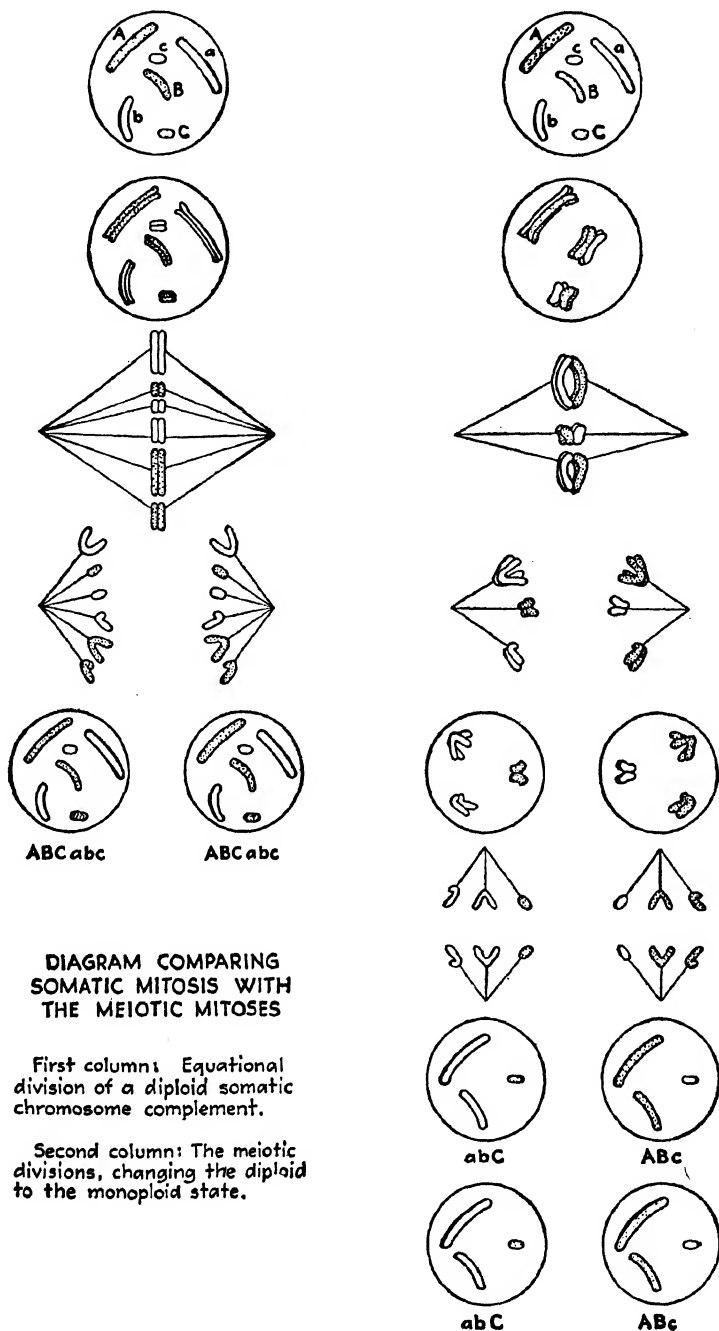


FIG. 28. — Comparison of somatic and meiotic mitosis. (From Sharp.) (65)

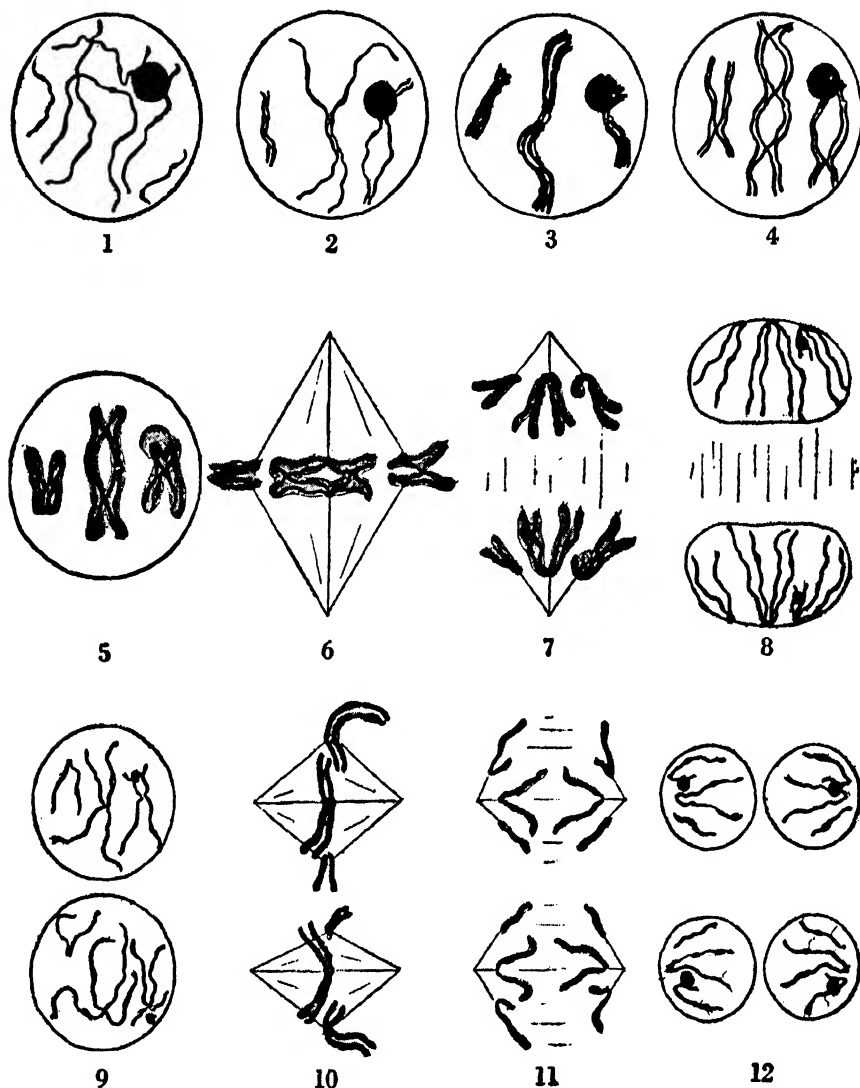


FIG. 29. — Diagram of meiosis. 1, first meiotic division, prophase, (leptonema stage). 2, do. showing synapsis (zygonema stage). 3, do. showing thickening of the chromosomes (pachynema stage). 4, do. showing formation of tetrads (diplonema stage). 5, do. showing condensation of matrix (diakinesis stage). 6, metaphase I. 7, anaphase I showing dyads. 8, telophase I. 9, second meiotic division, prophase showing dyads united at attachment points. 10, metaphase II. 11, anaphase II showing the separation of the chromatids which composed the dyads. 12, telophase II. Each of the four germ cells now has the haploid number of chromatids (chromosomes). (From Sharp.)

In the anaphase (Fig. 29, 7), the daughter chromosomes each possess two chromatids and are known as dyads. But there are two different ways of dividing a tetrad. In one case the two chromatids derived from one of the synaptic mates ($\frac{A}{A}$) might be separated from those derived from the other mate ($\frac{a}{a}$) in a reduction (disjunction) division. In the other, each dyad might contain one chromatid from each of the synaptic mates ($A : a$) as the result of an equation division.

The telophase (and prophase of the second meiotic division) sometimes is omitted if the second division succeeds the first immediately.

Second meiotic division. — If these omissions take place, each of the daughter 'cytes divides immediately, the chromosomes, still in the dyad condition, lining up on the spindles for the metaphase of the second meiotic division. But even if the telophase of the first and prophase of the second meiotic divisions are not omitted (Fig. 29, 8), it is obvious that the chromosomes arising in the prophase (Fig. 29, 9) are dyads and that they undergo no other longitudinal split. The anaphase of the second meiotic division (Fig. 29, 11) merely separates the two chromatids of each dyad from each other. The final result is that the four cells produced by the meiotic divisions (Fig. 29, 12) each have one chromatid from each tetrad or one-half the number of chromosomes found before meiosis took place. This is expressed in another way by saying that the number of chromosomes has been reduced from the diploid to the haploid (monoploid) number.

Here we must note that it makes no difference whether the first meiotic division divided a tetrad reductionally or equationally. The second division always distributes the two chromatids of each dyad into different cells. Each of the four daughter cells has one chromatid from each tetrad, and therefore one representative from either one of the two synaptic mates (A or a), but not from both.

Distribution of the chromosomes. — As each tetrad orients itself independently upon the spindle it is evident that it is a matter of chance which half of a tetrad, or of a dyad, goes to either pole of the spindle. Accordingly, if we had eight chromosomes, A, a, B, b, C, c, D, d , these would unite in synapsis to

form four double chromosomes, Aa , Bb , Cc , and Dd . These would form the four tetrads, $\frac{A : a}{A : a}$, $\frac{B : b}{B : b}$, $\frac{C : c}{C : c}$, and $\frac{D : d}{D : d}$. Following the two meiotic divisions (equation and reduction, regardless of their order), the mature germ cells would have four chromosomes (the haploid number), but only one representative of each synaptic pair. The possible combinations are 2^4 or 16, namely, $ABCD$, $ABCd$, $ABcd$, $Abcd$, $ABcD$, $AbCD$, $AbCd$, $aBCD$, $aBCd$, $aBcd$, $aBcD$, $abcD$, $abCD$, and $abCd$ (Fig. 30).

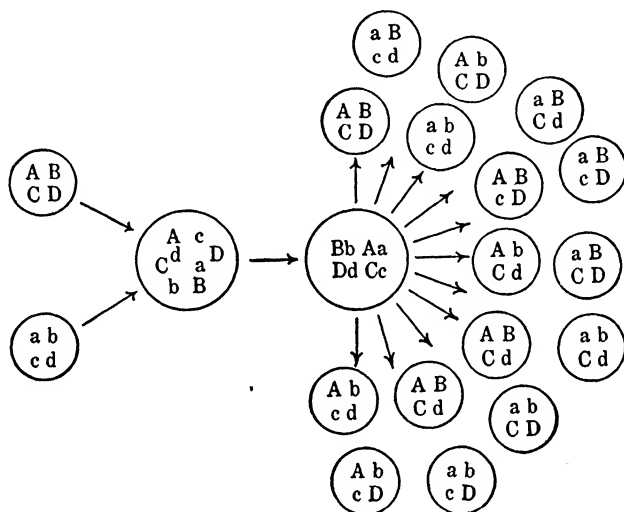


FIG. 30. — Showing the distribution of the chromosomes in fertilization and the following meiotic divisions. (After Wilson.)

Accordingly the number of different types of gametes which may be formed can be determined from the formula 2^n when n is the haploid number of chromosomes characteristic of the species.

The chromosomes in fertilization. — Evidently when the egg and sperm unite in fertilization, the pronucleus contributed by each contains the haploid number of chromosomes. In this way the diploid number characteristic of the species is restored. It is obvious that, unless the number had been reduced by meiosis, it would be doubled in each new generation.

In the second place, it is clear that each germ cell contributes a homologous set of chromosomes, and that in synapsis the chromosomes unite in homologous pairs. In the example referred to

above, chromosomes *A*, *B*, *C*, *D* came from one parent and *a*, *b*, *c*, *d* came from the other. We can now visualize each synaptic pair as consisting of one paternal and one maternal chromosome.

During meiosis the paternal and maternal chromosomes are sorted out into different assortments in the different germ cells. During fertilization these different assortments are brought together in random recombinations. We have said that in an animal with 8 chromosomes we might have 2^4 or 16 different classes of gametes. In random fertilization this number would be squared, so that there would be 4^4 or 256 possible combinations. Many of these would be duplicates, so that the exact number of different classes of zygotes according to their assortment of chromosomes would be 3^4 or 81.

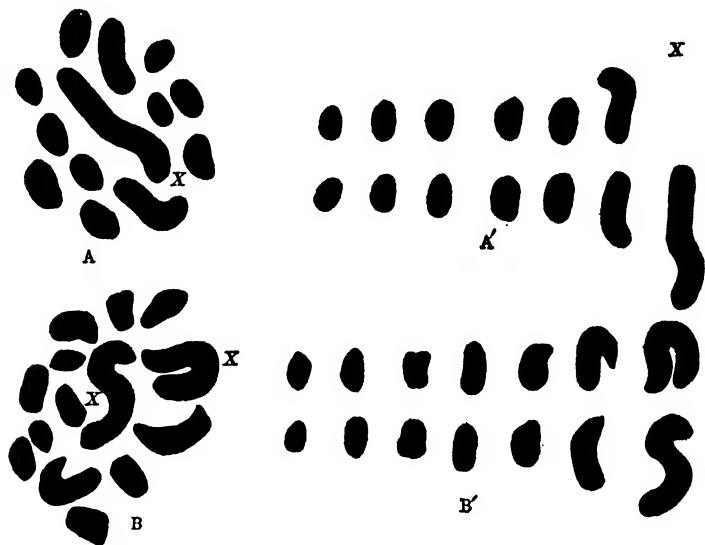


FIG. 31. — Chromosomes of *Protenor*. A, A', male diploid group. B, B', female diploid group. The X-chromosomes are indicated by X. (After Wilson.)

Sex chromosomes, X-O type. — In many animals, such as the insect *Protenor*, the male has one chromosome less than the female, the numbers in *Protenor* being 13 and 14, respectively (Fig. 31). If the synaptic pairs are assembled, it is clear that the male has six pairs of ordinary chromosomes (autosomes) and an extra one, the X-chromosome. The female has six pairs of autosomes and a pair of X-chromosomes. In the female the X-

chromosomes unite in synapsis, form a tetrad, and are segregated in the meiotic divisions so that every egg has a complete set of autosomes and one X-chromosome ($A + X$). In the male, on the other hand, the single X-chromosome has no synaptic mate and so goes on the spindle of the first meiotic division as a dyad, which is carried to one pole of the spindle entire. In the second meiotic division the dyad is divided as usual. The end result is that only half the spermatids receive an X-chromosome, and two classes of sperms are formed, either with or without an X-chromosome ($A + X$ or $A + 0$). If a sperm with an X-chromosome fertilizes the egg, the female combination ($2A + 2X$) is restored.

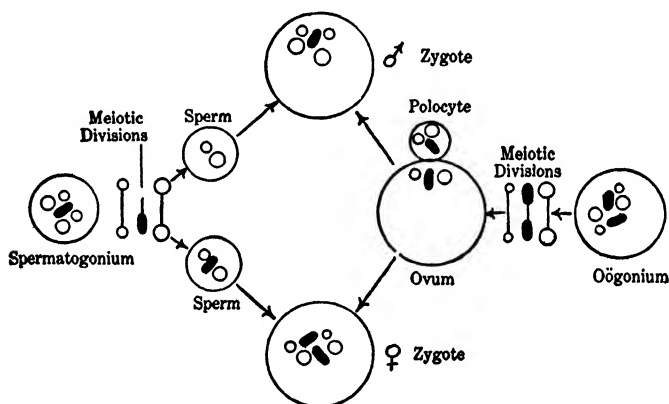


FIG. 32. — Diagram showing history of the X-chromosome during meiosis and fertilization. (After Wilson.)

If a sperm without the X-chromosome penetrates the egg, the male combination ($2A + X$) is formed (Fig. 32).

Sex chromosomes, X-Y type. — But the sexes do not always differ in chromosome number, for in many animals, like the insect *Lygaeus* (Fig. 33), the X-chromosome of the male is furnished with a synaptic mate which differs from it in size, form, and probably composition, and is therefore known as the Y-chromosome. The male forms a tetrad $\frac{X : Y}{X : Y}$, and the sperms therefore

have either an X-chromosome or a Y-chromosome. Fertilization by a sperm bearing the X-chromosome results in the development of a female ($2A + 2X$), whereas if a sperm bearing a

Y-chromosome enters the egg the embryo will give rise to a male ($2A + XY$).

Sex chromosomes, W-Z type. — As an exception to the general rule among the vertebrates, the birds have dissimilar sex chromosomes in the female. The cytological details are difficult to interpret but the theoretical explanation is that the female has two dissimilar sex chromosomes known as *W* and *Z*, while the male

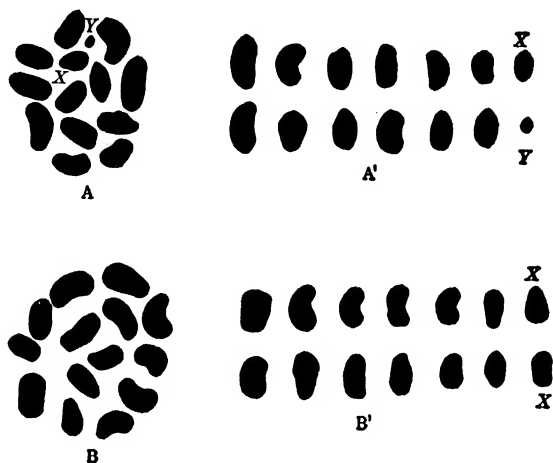


FIG. 33. — Chromosomes of *Lygaeus*. A, A', male diploid group. B, B', female diploid group. X and Y indicate the X- and Y-chromosomes respectively. (After Wilson.)

possesses two similar sex chromosomes of the *Z* type (Fig. 34B). In the meiosis of the oöcyte, therefore, a tetrad $\frac{W : Z}{\overline{W} : \overline{Z}}$ is formed, and the ovum receives either a *W*-chromosome or a *Z*-chromosome. The spermatocyte forms a tetrad $\frac{Z : Z}{\overline{Z} : \overline{Z}}$, and all sperms carry one *Z*-chromosome. In this group, therefore, it is the ovum which determines the sex of the embryo rather than the sperm. This explanation agrees with the data obtained from genetics.

CHROMOSOMES OF THE AMPHIOXUS. — The diploid number is 24.

CHROMOSOMES OF THE FROG. — The diploid number is 26, and the sex chromosomes of the male are of the *X-Y* type (Fig. 34A).

CHROMOSOMES OF THE CHICK. — The diploid number is 35 or 36. The sex chromosomes have not been positively identified, but genetic evidence indicates that the sex chromosomes of the female are of the *O-Z* or the *W-Z* type (Fig. 34B).

CHROMOSOMES OF MAN. — The diploid number, according to the most recent researches, is 48. The sex chromosomes are of the *X-Y* type (Fig. 34C). It is interesting to note that with 48 chromosomes the possible types of gametes number 2^{24}

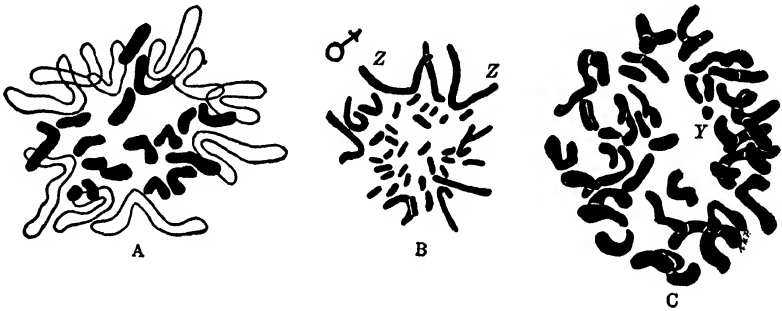


FIG. 34. — Metaphase plates of male diploid chromosome groups. A, frog (after Witschi). B, chick (after Hance). C, man (after Painter).

or 16,777,300, and that from these 3^{24} zygote-recombinations are possible.

B. THE GENES

It has already been said that the behavior of the chromosomes itself might suggest that these bodies are concerned with the transmission of hereditary characters. We shall now turn our attention to the laws of heredity as worked out by plant and animal breeders and learn how the data of genetics agree with the data of cytology.

The unit of genetics is the gene. These genes are arranged in linear order in the chromosomes, presumably bound together by the chromonemata, and possibly identified with the chromomeres. They exist in great numbers; in the fruitfly *Drosophila* it is estimated that there are between 2000 and 3000. Ordinarily ultramicroscopic, it has been reported recently by Belling (1930) and by Bridges (1934) that they have been able to identify these units in material of exceptionally favorable nature. The genes are known by the effects their presence induces, and named according to the most obvious of these effects. Thus the *Drosophila*

has a gene for (or a gene which induces among other effects) the normal type of wing. But there have arisen, among the millions of fruitflies raised by geneticists, some with abnormal types of wings, such as a vestigial wing. In this case there is said to be a gene for (or a gene which induces among other effects) the vestigial type of wing.

Dominance. — Among the original discoveries of Mendel was the fact that, if two organisms with alternative characters were mated, the offspring would show either one or the other of the characters concerned. This is known as the law of dominance. When a *Drosophila* with normal wings is mated to one with vestigial wings, all the offspring have the normal type of wing (Fig. 35). Therefore the gene for normal is said to be dominant to the gene for vestigial, which, conversely, is said to be recessive to the gene for normal.

It is customary among geneticists to use the initial letter of the name for the abnormal character as a symbol for the gene inducing its appearance, as well as a symbol for the gene inducing the alternative (allelomorphic) normal character. The two are distinguished by using a capital letter for the dominant gene, a lower-case letter for the recessive gene. In this case, then, the symbol of the gene for vestigial is v , and the symbol of the gene for normal is V .

Every adult has two haploid sets of chromosomes, and therefore a pair of every kind of chromosome. If both members of a pair have the same gene (vv or VV) they are said to be homozygous; but if one chromosome has the dominant, and the other has the recessive gene (Vv), they are said to be heterozygous.

The individuals that are mated together in the first instance are known as the parental generation (P_1); their offspring are known as the first filial generation (F_1); the next generation is the second filial generation (F_2); and so on.

Segregation. — In the experiment where a normal long-winged fly was mated with a vestigial-winged fly, the long-winged parent must have had two chromosomes each containing the dominant gene V , for all the offspring (F_1) showed this dominant character. The vestigial-winged parent must have had two chromosomes containing the recessive gene v . In the maturation of the gametes all the sperms received V , while the eggs all received v .

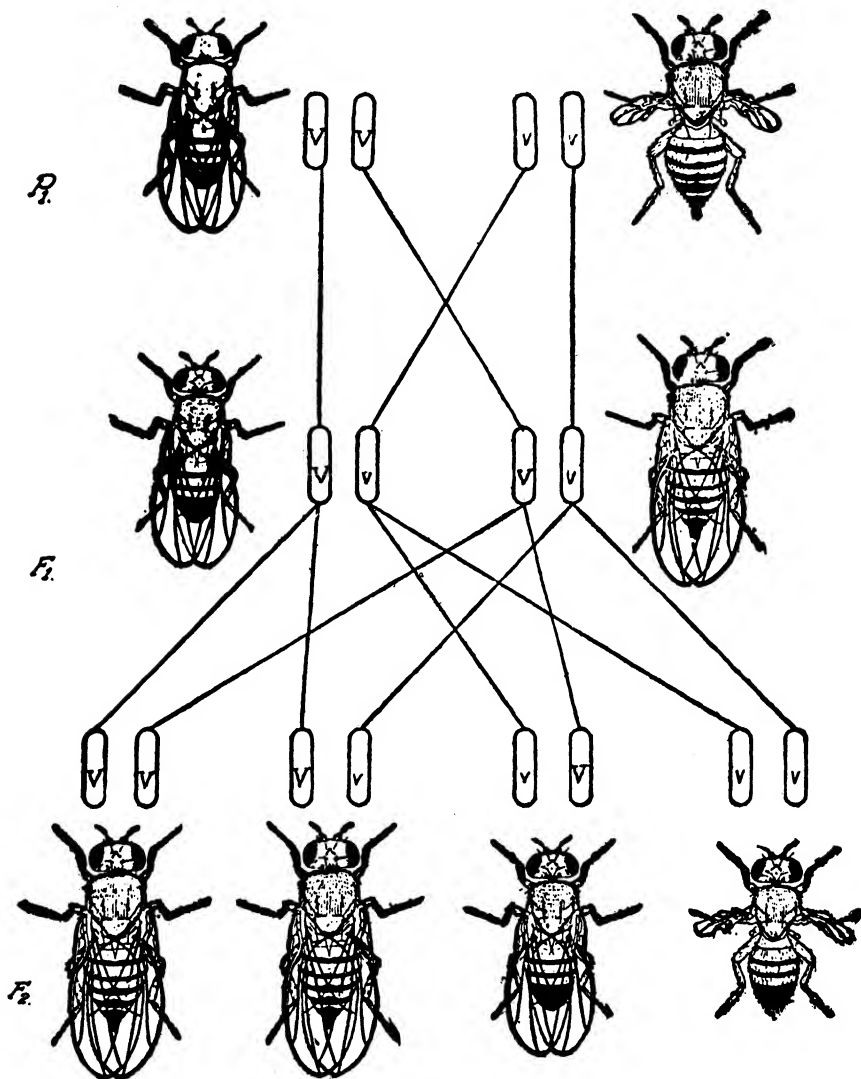


FIG. 35. — Diagram to show the effects of crossing two flies differing in respect to one pair of genes. V is used for the dominant gene for the normal character long wings; v is used for the recessive mutant gene for vestigial wing. (From Curtis and Guthrie, after Morgan *et al.*)

The F_1 flies have the genetic constitution Vv , that is to say, they are heterozygous. When they are mated to each other the eggs will receive a chromosome containing the gene V or the gene v , and the same is true of the sperm.

The F_2 flies will consist of three genetic groups (genotypes) because of random fertilization, namely, homozygous long-winged flies (VV), heterozygous long-winged flies (Vv), and vestigial-winged flies (vv). From Fig. 36, it will be seen that the ratio will be one homozygous long-winged fly to two heterozygous long-winged flies, and to one vestigial-winged fly. Or one may say that there are two recognizable classes of adults (phenotypes), in the ratio of three long-winged flies to one vestigial-winged fly. This is the famous Mendelian ratio applied to the inheritance of

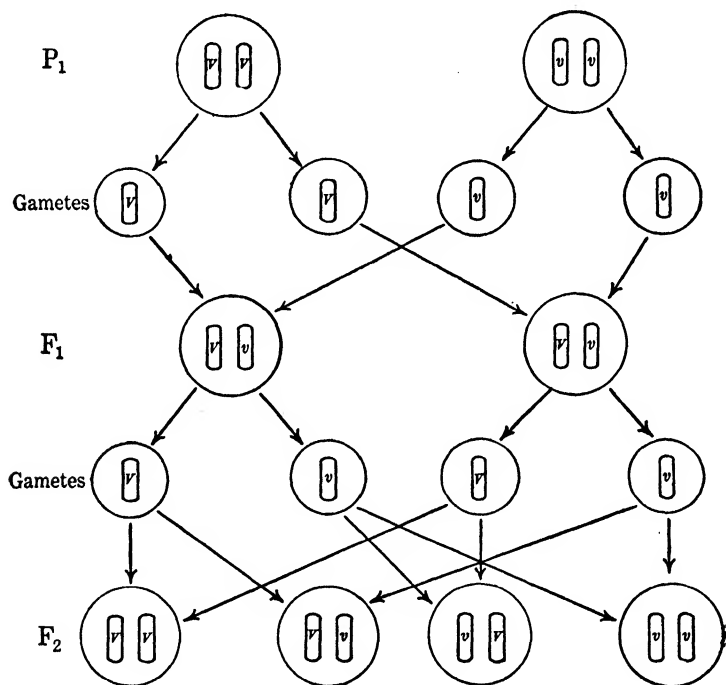


FIG. 36. — Diagram to show the segregation of the genes caused by the distribution of the chromosomes to the gametes and zygotes of the F_1 and F_2 generations. V , and v as before.

one pair of allelomorphous characters, or as we should say today, to one pair of genes.

Evidently Mendel's law of segregation may be stated in terms of the gene theory as follows: *allelomorphic genes are segregated during maturation into different gametes.*

Assortment. — It is an amazing coincidence that Mendel studied the inheritance of seven pairs of allelomorphous characters in

the edible pea, a species which has seven pairs of chromosomes, and that the genes for each pair of characters were located in a different pair of chromosomes.

When a *Drosophila* with vestigial wings and normal gray body color is mated to a fly with normal long wings and ebony body color, the F_1 flies are gray-bodied and long-winged. Evidently the gene for gray body (E) is dominant to the gene for ebony body (e). That the genes for these characters are independent of those affecting wing length is shown when the hybrid F_1 flies are mated together. Four classes of phenotypes result in the F_2 generation: 9 long-winged, gray-bodied; 3 long-winged, ebony-bodied; 3 vestigial-winged, gray-bodied; and 1 vestigial-winged, ebony-bodied. This ratio of 9 : 3 : 3 : 1 breaks down to 3 long to 1 vestigial, and 3 gray to 1 ebony, demonstrating mathematically that two pairs of factors are involved.

It is evident that the problem involves the segregation of two pairs of chromosomes (Fig. 37). The genetic constitutions of the P_1 flies were $vvEE$ and $VVee$, respectively. The gametes receive one chromosome from each pair of synaptic mates, so the genetic constitution of the eggs is vE and that of the sperms Ve (or vice versa).

The F_1 flies have the formula $VvEe$, and their gametes, because the chromosome pairs are assorted independently, will belong to four classes: VE , Ve , vE , and ve .

The F_2 flies as seen from the checkerboard diagram will fall into 16 combinations, which by canceling the duplicates reduce to 9 genotypes ($VVEE$, $VVEe$, $VvEE$, $VvEe$, $VVee$, $Vvee$, $vvEE$, $vvEe$, and $vvee$), and 4 phenotypes as listed above.

Mendel's law of assortment may be phrased in terms of gene theory as follows: *different pairs of allelomorphic genes when located in different pairs of chromosomes are assorted independently during maturation into different gametes.*

It may be noted that if n stands for the number of pairs of genes located in different pairs of chromosomes, then 2^n represents the number of gamete classes formed by the F_1 generation; 2^n represents the number of phenotypes in the F_2 generation; 3^n the number of genotypes in the F_2 generation; and 4^n the number of combinations in the Punnett square. The number of individuals in each phenotype is obtained by expanding the 3 : 1 formula as follows: (3 : 1), (9 : 3 : 3 : 1), (27 : 9 : 9 : 9 : 3 : 3 : 3 : 1)

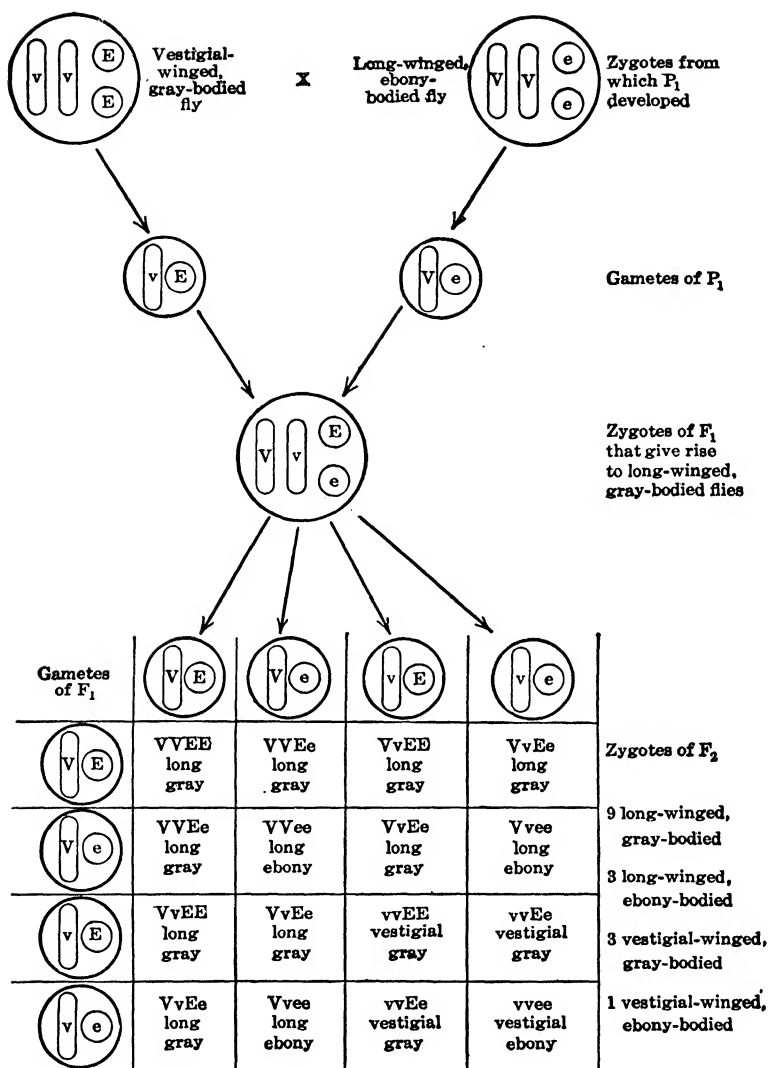


FIG. 37. — Diagram to show the assortment of two pairs of genes due to the distribution of two pairs of chromosomes. E , gene for gray body; e for ebony body; V , and v as before. (From Curtis and Guthrie.)

Linkage. — The characters with which Mendel worked segregated freely, showing that their genes were not borne in the same chromosome. Later studies have shown that some characters do not segregate, and this leads to the assumption that their genes are carried in the same chromosome and therefore are inherited together.

When a *Drosophila* with gray body color and long wings is mated to one with black body color and vestigial wings, the F_1 flies are gray-bodied and long-winged. Note that the gene for black (b) will act very differently from the gene for the similar color ebony (e). If the F_1 flies are bred together a very confusing ratio appears in the F_2 generation: practically all the flies are gray-bodied and long-winged or black-bodied and vestigial-winged like the P_1 generation, but there are only a few individuals representing the other classes we might expect under Mendel's law of assortment. If we make a reciprocal cross between a long-winged black-bodied fly and a vestigial-winged gray-bodied fly, the F_2 flies are all of these two (P_1) types with few exceptions. This continued association of two genes through several generations is called linkage and suggests that the associated genes are located in the same chromosome.

This theory may be tested by back-crossing (Fig. 38) a male of the F_1 generation ($BbVv$) to a double recessive female ($bbvv$). All her eggs will have the recessive genes (bv). We can then test the constitution of the sperm by examining the progeny of this cross (here called F_2 for convenience), for all the F_2 flies must have the genes (bv) from the mother. The flies of this generation are either gray-bodied and long-winged ($BbVv$) or black-bodied and vestigial-winged ($bbvv$). This seems to show that the genes B and V were located in one chromosome while b and v were located in the synaptic mate.

Crossing over. — Now for the exceptional (cross-over) flies noted above. There is no crossing over in the maturation of the male F_1 fly, but how about the female? When we mate (Fig. 39) a female F_1 fly ($BbVv$) to a double recessive male ($bbvv$), the progeny (F_2) fall into four classes: $41\frac{1}{2}$ per cent gray-bodied and long-winged ($BbVv$), $41\frac{1}{2}$ per cent black-bodied and vestigial-winged ($bbvv$), $8\frac{1}{2}$ per cent gray-bodied and vestigial-winged ($Bbvv$), and $8\frac{1}{2}$ per cent black-bodied and long-winged ($bbVv$).

Obviously there has been an exchange of some sort between the chromosomes of the female F_1 fly. Both cytological and experi-

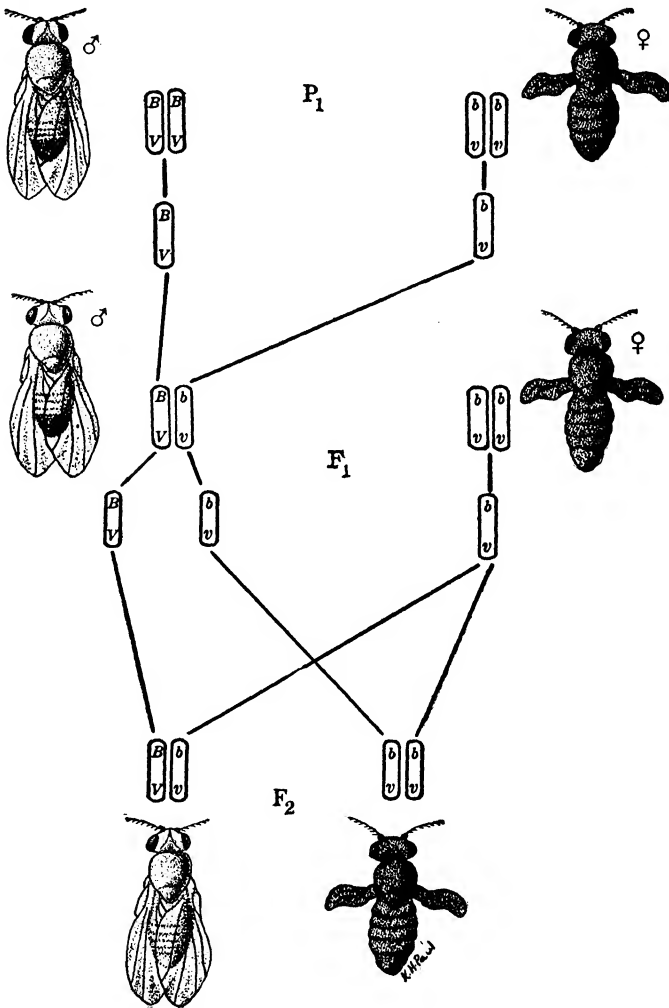


FIG. 38. — Diagram to show the inheritance of two pairs of genes when located in one pair of chromosomes, (linkage). In this case the male F_1 fly is back-crossed to a double recessive female. B , gene for gray body; b , for black body; V , and v as before. (After Morgan.)

mental evidence seem to indicate that this crossing over takes place in the prophase of the first meiotic division (Fig. 29, 4). Although there are still difficulties in determining exactly how the crossing over takes place between the four strands, it is gener-

ally agreed that the actual crossing over takes place between two of them. The idea of linkage between genes in the same chromo-

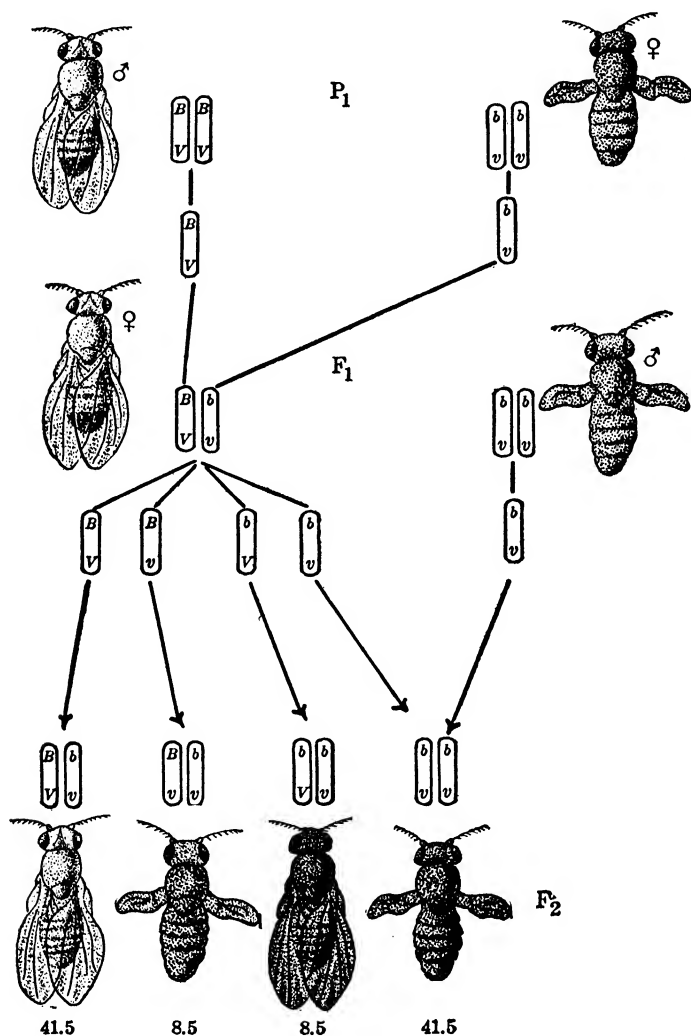


FIG. 39. — Diagram to show the inheritance of two pairs of genes when located in one pair of chromosomes between which crossing over takes place. In this case the female *F*₁ fly is back-crossed to a double recessive male. Symbols as in Fig. 38. The figures at the bottom of the illustration indicate the percentage of each phenotype in the entire hatch. (After Morgan.)

some suggested the idea that the genes form a longitudinal series in each chromosome. This is supported by the behavior of the

chromosomes in ordinary somatic mitosis, in synapsis, and in crossing over (Fig. 40).

Finally Sturtevant (1913) suggested that the percentage of crossing over between two pairs of linked genes might represent a function of the distance between the loci of the genes in the chromosome. Accordingly, maps have been constructed, by Morgan and his co-workers, on the general assumption that one per cent of cross-overs is represented on the map by a distance of one unit between the genes involved. Without going into further details of the methods used in constructing these maps, for there

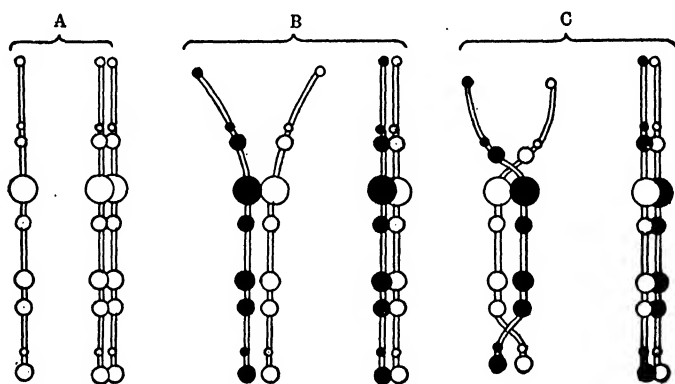


FIG. 40. — Diagram to illustrate: A, splitting of a chromosome in somatic mitosis; B, union of two chromosomes in synapsis; C, union of two chromosomes in synapsis accompanied by crossing over. (After Wilson.)

are many complicating factors, a glance at the accompanying chart (Fig. 41) will show the progress that has been made in this direction.

Sex-linked inheritance. — One of the most striking evidences that genes are borne in the chromosomes is afforded by what is known as sex-linked (criss-cross) inheritance. This is illustrated in *Drosophila* by the inheritance of white eye color, an allelomorph of red, the normal eye color. If a white-eyed male is mated with a red-eyed female (Fig. 42), the F_1 flies of both sexes will have red eyes. But if these F_1 flies are bred together the F_2 generation will be made up of red-eyed females, (50 per cent) red-eyed males (25 per cent), and white-eyed males (25 per cent). It looks at first like an ordinary 3 to 1 Mendelian ratio, except for this curious distribution of eye color in the two sexes.

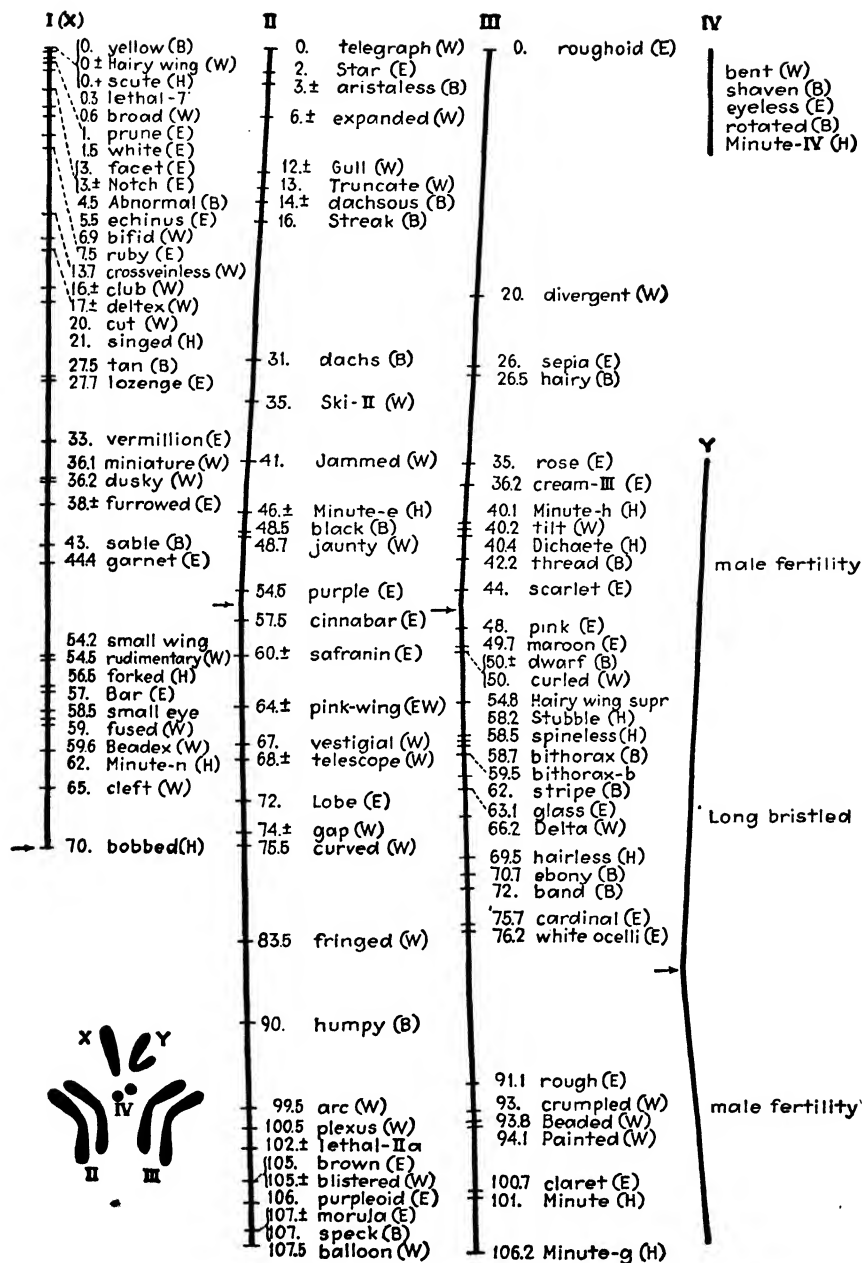


FIG. 41. — The chromosomes of *Drosophila melanogaster* and map showing the positions of many genes as determined from cross over ratios. Letters in parentheses indicate part of body affected: B, body; E, eye; H, hair; W, wing. Arrows indicate position of attachment point. All genes in IV are closely linked. The exact position of genes in Y still undetermined. (From Sharp after Morgan *et al.* 1925 and Stern 1929.)

In the reciprocal mating, a red-eyed male to a white-eyed female (Fig. 43), the F_1 generation is made up of red-eyed females

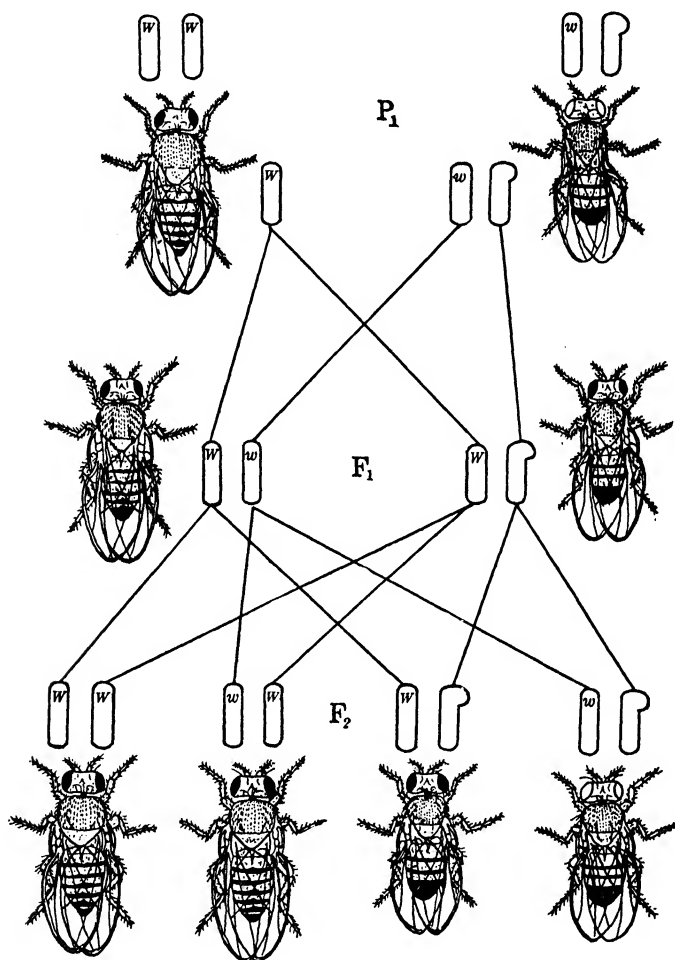


FIG. 42. — Diagram to show the inheritance of one pair of genes when located in the X-chromosome (sex-linkage). W , gene for red eye (dominant, normal); w , gene for white eye (recessive, mutant). The empty hook-shaped chromosome represents the Y-chromosome. N. B. In the text the X-chromosome, when bearing w , the gene for white eyes, is designated by a small x . In this cross, red-eyed female is mated to a white-eyed male. (After Morgan *et al.*)

and white-eyed males (criss-cross inheritance). When these F_1 flies are bred together, there are four classes of flies in the F_2 generation: red-eyed males and females, and white-eyed males

and females (25 per cent in each class). This is not a Mendelian ratio, but it can be explained on the assumption that the gene for

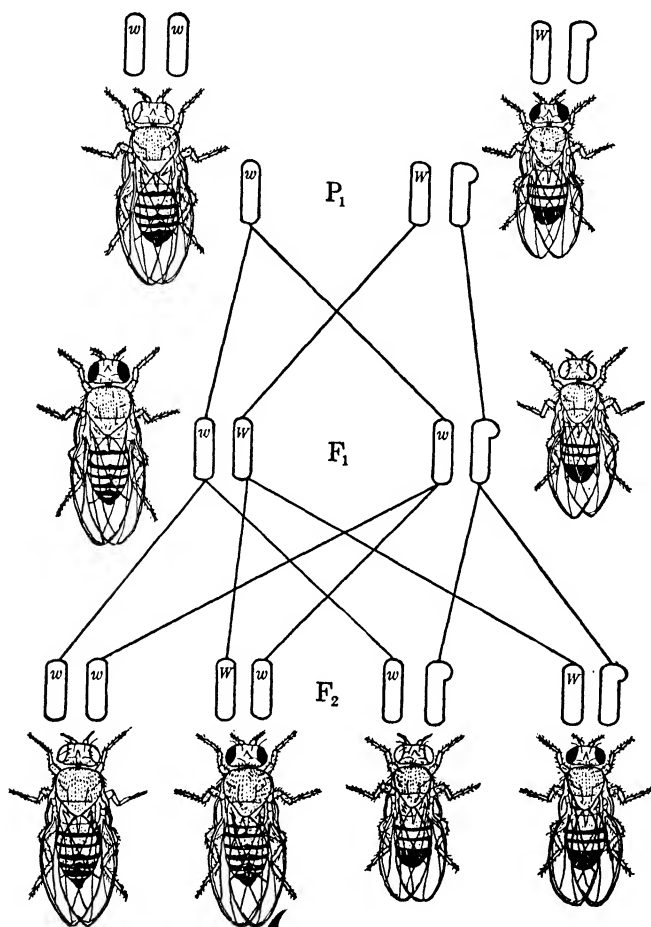


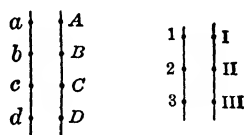
FIG. 43. — The reciprocal cross to that shown in Fig. 42. A white-eyed female is mated to a red-eyed male. Symbols as in Fig. 42. (After Morgan *et al.*)

white eye color (and its allelomorphs, of which there are several) is located in the X-chromosome.

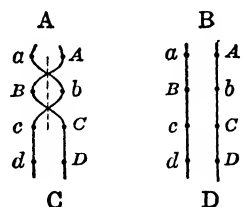
Let us use the symbol X for an X-chromosome bearing a gene for red, x for an X-chromosome bearing a gene for white, and Y for the Y-chromosome. In the first genetic experiment, formulas for the parental generation are XX (red female) and xY (white male). All the eggs receive an X , the sperms either

x or Y . Consequently the F_1 generation is made up of flies with the formula Xx (heterozygous red female) and XY (red male). The eggs of this generation receive X or x , the sperms X or Y .

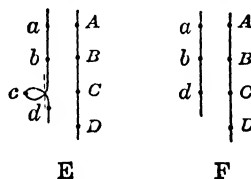
Two pairs of homologous chromosomes showing positions of allelomorphic genes.



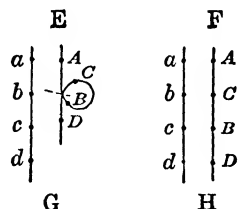
Crossing over: The chromosomes of the pair shown in A may twist about one another as in C and break in the plane of the dotted line so that comparable sections are exchanged as shown in D.



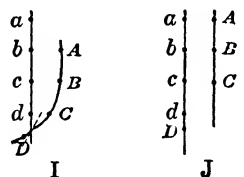
Deletion: One member of the chromosome pair shown in A may twist on itself as in E and break in the plane of the dotted line so that an internal section containing gene c is lost, or deleted, as shown in F.



Inversion: One member of the chromosome pair shown in A may twist on itself as in G and break in the plane of the dotted line so that the section containing genes B and C is inverted as shown in H.



Duplication and Deficiency: If one member of the chromosome pair shown in A comes to lie across the other as shown in I and a break occurs in the plane of the dotted line, the chromosome on the left in J will have a duplication and contain both gene d and gene D , while the chromosome on the right will have a deficiency of the section containing gene D .



Translocation: One member of the chromosome pair shown in A may come to lie across one member of the chromosome pair shown in B, as seen in K. If a break occurs in the plane of the dotted line, sections of non-homologous chromosomes are exchanged, or translocated, as shown in L.

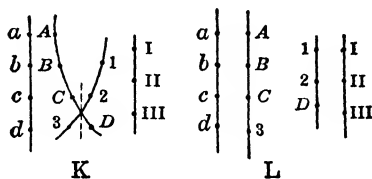


FIG. 44. — Diagrams to show crossing over and various chromosomal aberrations. (From Curtis and Guthrie.)

So the F_2 generation is composed of flies with the following combinations: XX (homozygous red females), Xx (heterozygous red females), XY (red males), and xY (white males).

In the other experiment the parental formulas are xx (white female) and XY (red male). The eggs receive an x , the sperm X or Y . Hence there are two classes in the F_1 generation, xX (heterozygous red females) and xY (white males). The eggs receive either an x or an X , the sperms receive either x or Y . The four combinations possible in the F_2 generation are xX (heterozygous red female), xx (white female), xY (white male), and XY (red male).

Chromosomal aberrations. — Crossing over takes place between the two X-chromosomes, but apparently not between the X-chro-

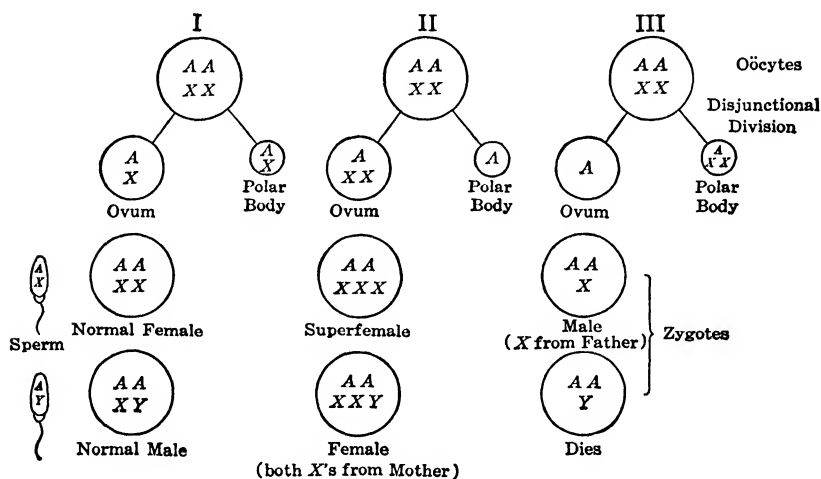


FIG. 45. — Diagrams showing I, normal disjunction of X-chromosomes in oögenesis, and fertilization by two types of sperms; II, non-disjunction, both X-chromosomes remaining in egg; III, non-disjunction, both X-chromosomes passing to polocyte. A, one haploid set of autosomes. (From Curtis and Guthrie.)

mosome and the Y-chromosome in *Drosophila*. We have already noted the fact that in this little fruitfly crossing over does not take place in the male.¹ But crossing over by no means exhausts the possibility of effecting new combinations of genes by the behavior of the chromosomes during the maturation of the germ cells. Exact as the mechanism of meiosis may seem, many possi-

¹ There is some recent evidence to show that such crossing over can be induced by high temperatures.

bilities of disturbance have been discovered by genetic and cytological methods.

The accompanying diagram (Fig. 44) illustrates graphically some of the aberrations which may take place during meiosis. These result in the appearance of unexpected individuals with new combinations of genes

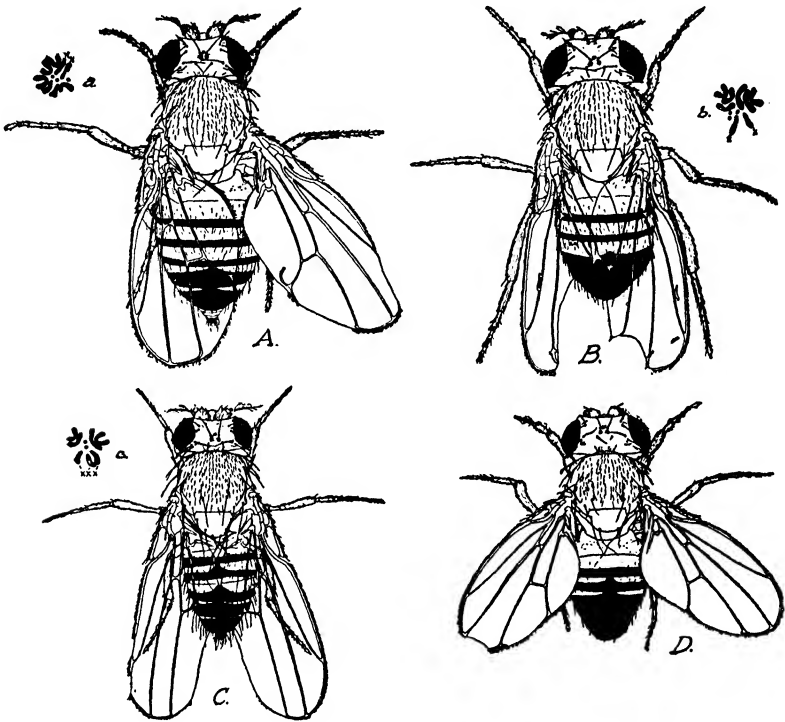


FIG. 46. — Intersexes and supersexes in *Drosophila*, occurring in the progeny triploid females. A, female-type intersex. B, male-type intersex. C, superfemale. D, supermale. a, b, and c are the chromosome groups characteristic of A, B, and C respectively. (From Curtis and Guthrie, after Morgan *et al.*)

Non-disjunction. — A special type of chromosomal aberration is one in which the two members of the synapctic pair may fail to separate during the meiotic divisions, so that one egg receives, for example, two X-chromosomes ($A + 2X$), while another receives none (A) (Fig. 45). When fertilized by a sperm with an X-chromosome, the egg with two X-chromosomes, if it develops into an adult, will be a superfemale ($2A + 3X$) differing markedly from her sisters (Fig. 46C). When fertilized by a sperm with a

Y-chromosome, the egg without any X-chromosomes ($2A + Y$) dies. The other possible combinations are shown in the diagram.

In some cases all the chromosomes fail to disjoin so that an egg receives a diploid set of chromosomes ($2A + 2X$). When fertilized by an $A + X$ sperm it becomes a triploid female ($3A + 3X$). The eggs formed by these triploid females may have the formula $2A + X$ or $A + 2X$. If an egg of the first type ($2A + X$) is fertilized by sperm carrying an X-chromosome ($A + X$), the zygote will have the formula $3A + 2X$. Such a zygote develops into an abnormal fly known as an intersex (Fig. 46A), male in some respects and female in others. Superfemales ($2A + 3X$) may also arise from the egg of the second type ($A + 2X$) being fertilized by an $A + X$ sperm. Supermales ($3A + XY$), on the other hand, arise from the fertilization of a $2A + X$ egg by an $A + Y$ sperm (Fig. 46D). It would appear from these formulas as though the determination of sex depended on some sort of ratio between the genes in the X-chromosomes and the autosomes, and

Bridges (1921) has formulated a theory of genic balance to account for the observed results.

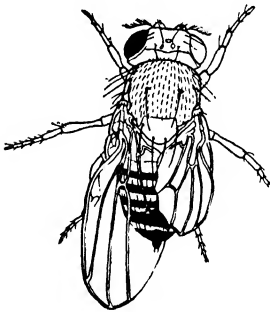


FIG. 47. — Gynandromorph in *Drosophila melanogaster*. Note eosin eye and miniature wing on right as compared to red eye and long wing on left. This fly is male on the right side and female on the left. (After Morgan and Bridges.)

Gynandromorphs. — Intersexes must not be confused with gynandromorphs, which are individuals with one part of the body male and the rest female. Bilateral gynandromorphs in *Drosophila* (Fig. 47) arise from female zygotes ($2A + 2X$), but during the first cleavage division one of the X-chromosomes is lost on the mitotic spindle. The result is that one of the daughter cells has the female complex ($2A + 2X$) while the other has the male complex ($2A + X$). Sometimes such an aberration takes place in a later cleavage division so that there is only a small area of male cells.

Teratology. — All students of embryology are familiar with the fact that development does not always proceed normally. Abnormal embryos are known as monsters, and their study forms the subject matter of the embryological subscience known as teratology. It is clear from the sections

just preceding that many of these monsters must be due to chromosomal aberrations with consequent disturbance of the genic balance. Others, as will be noted in Chapter VII, are due to environmental factors.

Mutations. — So far we have considered the genes as though they were immutable. But the question naturally arises as to the origin of the genes which are allelomorphic to the so-called normal genes. In *Drosophila* the abnormal genes, or mutants as they are called, arose in laboratory cultures. It has been discovered that the rate of mutation, i.e., the number of mutants arising in a given number of flies, may be increased by high temperatures (Plough) and by irradiation (Müller). When one of these genes is altered in any way to become a mutant, the course of development is disturbed. Most mutant genes disturb the course of development so greatly as to cause death (lethal mutants). A smaller number produce visible changes when present in each chromosome of the synaptic mates (recessive mutants). A few produce visible changes if contained in a single chromosome (dominant mutants). Accordingly, every species of animals contains a certain number of mutant genes (400 in *Drosophila*). As these enter into new genetic combinations according to the behavior of the chromosomes in meiosis and fertilization, they give rise to individual differences in development. But the greater number of stable or non-mutant genes holds development true to the specific type.

One of the outstanding problems in experimental embryology still awaiting solution is the question how the genes actually determine the course of development. But the modern student of embryology accepts the general theory that it is the complement of genes, from the egg and sperm respectively, which initiates and largely controls the development of the individual.

SUMMARY

The egg and sperm are the material contributions of the parents to the new individual. The equivalent structures of the egg and the sperm are their nuclei. Each nucleus contains the haploid number of chromosomes. The fertilized egg has two haploid sets, or the diploid number. In somatic mitosis the chromosomes are split longitudinally and divided equally among the daughter

cells, so that each daughter cell contains an assortment precisely equivalent to that of its sister cell and the mother cell. In the course of the meiotic divisions the diploid number of chromosomes is reduced to one haploid set. This is accomplished through the union of the homologous members of the two sets in synapsis. Each synaptic pair forms a tetrad of four chromatids, the members of which are distributed independently among the mature germ cells. In this way different classes of gametes are formed with varying chromosomal complexes.

The chromosome is built up from a thin thread, the chromonema, which binds together the genes, the units of heredity, provisionally located at nodes of the chromonema called chromomeres. These genes, ordinarily ultra-microscopic, are self-reproducing units which seem to accelerate definite chemical reactions without losing any of their own substance in the process. The course of development is largely controlled by the activities of these genes. These activities may be disturbed during meiosis by chromosomal aberrations, thus altering the genic balance and modifying the course of development, in some cases so much as to cause death. The genic balance may also be altered by point mutations or changes in the constitution of an individual gene recognizable through the effects produced.

Either aberrations or point mutations when not lethal may be transmitted in heredity. The distribution of these aberrant chromosomes or mutant genes in meiosis and fertilization is the material basis for heritable differences arising in the course of development of individuals belonging to the same species.

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CHAPTER V

CLEAVAGE AND THE GERM LAYERS

The fertilized egg (zygote) is a complete and balanced cell. It has two entire sets of chromosomes, each with a full complement of genes, one set from each parent. These nuclear elements are contained in a cell body whose cytoplasm is principally maternal in origin and which has a definite organization as indicated by its polarity. We are now to examine the way in which the embryo develops from the fertilized egg.

It is customary to distinguish three steps in the early development of the embryo. First is the period of cleavage in which the egg undergoes a number of mitotic cell divisions at each of which the number of cells (blastomeres) increases while the size of the cells decreases. The period ends with the embryo in the form of a blastula, a sphere or disc in which the blastomeres are not stratified into different layers.

Second comes the period of gastrulation in which the blastomeres arrange themselves into an outer and inner layer of cells, known as ectoderm and endoderm, respectively. This two-layered embryo is called a gastrula.

Third is the period in which a middle layer, including the mesoderm and the notochord, is formed between the ectoderm and endoderm. Although this layer sometimes develops during gastrulation, it is customary to distinguish a period of mesoderm (chorda-mesoderm) formation. This distinction is not always valid, nor is it important, for, as will be seen, the material which is to form the middle germ layer can sometimes be distinguished in gastrulation, cleavage, or even in the fertilized egg.

A. CLEAVAGE

As there are different types of eggs according to the amount and distribution of the yolk, so there are different types of cleavage according to the pattern formed by the dividing egg.

Rules of cleavage. — Certain rules have been formulated to express the simpler geometrical relationships of the blastomeres.

The first are those of Sachs: (1) cells typically tend to divide into equal parts; (2) each new plane of division tends to intersect the preceding one at right angles. Sachs's rules are supplemented, and to some extent explained, by those of Hertwig: (1) the typical position of the nucleus (and hence of the mitotic figure) tends towards the center of its sphere of influence, i.e., of the protoplasmic mass in which it lies; (2) the axis of the spindle typically lies in the longest axis of the protoplasmic mass, and division therefore tends to cut this axis transversely.

Methods of cleavage. — The rate of division is governed by the rule of Balfour: the rate of cleavage is inversely proportional to the amount of yolk present. This leads to a distinction between two types of cleavage. In the first type the cleavage

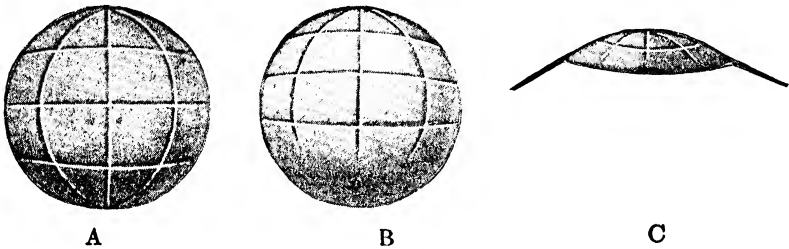


Fig. 48. — Diagram to show main types of cleavage in vertebrates. A, equal holoblastic. B, unequal holoblastic. C, meroblastic.

planes divide the egg completely into separate blastomeres. This is known as holoblastic cleavage, and is characteristic of isolecithal and moderately telolecithal eggs. In the second type the cleavage planes do not pass through the yolk and so the separate blastomeres come to lie upon a mass of undivided yolk. This is known as meroblastic cleavage and is typical of extremely telolecithal eggs. It is generally true that isolecithal eggs have equal holoblastic cleavage (Fig. 48A). Moderately telolecithal eggs have unequal holoblastic cleavage (Fig. 48B), and extremely telolecithal eggs have meroblastic cleavage (Fig. 48C).

Cell lineage. — It must not be thought that cleavage results in a mass of identical blastomeres. Painsstaking examination of dividing eggs has shown that in the normal development of favorable material the origin and fate of every blastomere can be determined accurately. The genealogical history of the blasto-

meres is known appropriately as cell lineage. One of the most clean-cut examples, in forms allied to the vertebrates, is the cell lineage of the tunicate *Styela* (*Cynthia*), worked out by Conklin in 1905. The accompanying diagram (Table 6) shows the cell lineage up to the 32-cell stage with the ultimate fate of each of the blastomeres.

In reading this chart the student should understand the system used in naming the blastomeres, which is illustrated most easily

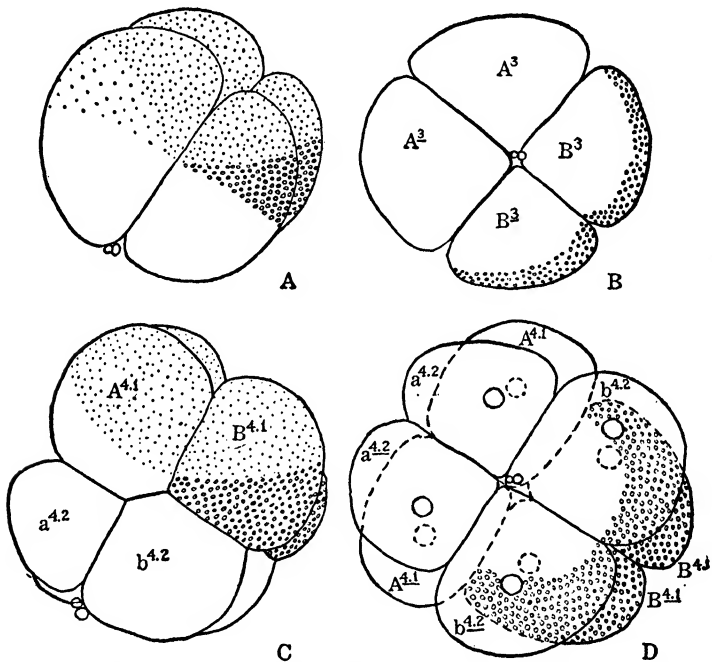


FIG. 49. — Cleavage of *Styela* (*Cynthia*) egg. A, 4-cell stage from left side. B, same stage from animal pole. C, 8-cell stage from left side. D, same stage from animal pole. For explanation of lettering see text. (From Richards, after Conklin.)

by means of the 8-cell stage (Fig. 49). The blastomeres which will give rise to structures on the right side of the embryo are underlined. The blastomeres formed at the animal hemisphere are in lower-case letters; those at the vegetal hemisphere are in capital letters. Those formed at the antero-dorsal side of the embryo are given the designation A or a; those at the postero-ventral side are named B or b. The first exponent is the number of the cell generation, counting the fertilized egg as the first

generation, the blastomeres of the first cleavage as the second generation, etc. The exponent after the decimal point indicates whether the cell is in the first, second, third, etc., row from the vegetal pole. Thus the cell labelled $A^{4.1}$ is antero-dorsal, left side, vegetal hemisphere, of the fourth generation, and in the row next to the vegetal pole.

TABLE 6

CELL LINEAGE OF <i>Styela</i> (<i>Cynthia</i>) AFTER CONKLIN (1905)						
(1st)	(2nd)	(3rd)	(4th)	(5th)	(6th)	Generation
(1)	(2)	(4)	(8)	(16)	(32)	Number of cells
Egg	$\overline{AB^2}$ (Left)	A^3	$a^{4.2}$	$\overline{a}^{5.4}$	$\overline{a}^{6.8}$	Ectoderm (epidermis)
				$\overline{a}^{5.3}$	$\overline{a}^{6.7}$	" "
					$\overline{a}^{6.6}$	" "
			$A^{4.1}$	$\overline{A}^{5.2}$	$\overline{A}^{6.4}$	Chorda-neural plate
				$\overline{A}^{5.1}$	$\overline{A}^{6.3}$	Endoderm
					$\overline{A}^{6.2}$	Chorda-neural plate
		B^3	$b^{4.2}$	$\overline{b}^{5.4}$	$\overline{b}^{6.8}$	Ectoderm
				$\overline{b}^{5.3}$	$\overline{b}^{6.7}$	" "
					$\overline{b}^{6.6}$	" "
			$B^{4.1}$	$\overline{B}^{5.2}$	$\overline{B}^{6.4}$	Mesoderm (gray crescent)
				$\overline{B}^{5.1}$	$\overline{B}^{6.3}$	" " "
					$\overline{B}^{6.2}$	" (yellow ")
		$\underline{AB^2}$ (Right)	\underline{A}^3	$\underline{\overline{a}}^{5.4}$	$\underline{\overline{a}}^{6.8}$	Ectoderm (epidermis)
				$\underline{\overline{a}}^{5.3}$	$\underline{\overline{a}}^{6.7}$	" "
					$\underline{\overline{a}}^{6.6}$	" "
			$\underline{A}^{4.1}$	$\underline{\overline{A}}^{5.2}$	$\underline{\overline{A}}^{6.4}$	Chorda-neural plate
				$\underline{\overline{A}}^{5.1}$	$\underline{\overline{A}}^{6.3}$	Endoderm
					$\underline{\overline{A}}^{6.2}$	Chorda-neural plate
		\underline{B}^3	$\underline{b}^{4.2}$	$\underline{\overline{b}}^{5.4}$	$\underline{\overline{b}}^{6.8}$	Ectoderm
				$\underline{\overline{b}}^{5.3}$	$\underline{\overline{b}}^{6.7}$	" "
					$\underline{\overline{b}}^{6.6}$	" "
			$\underline{B}^{4.1}$	$\underline{\overline{B}}^{5.2}$	$\underline{\overline{B}}^{6.4}$	Mesoderm (gray crescent)
				$\underline{\overline{B}}^{5.1}$	$\underline{\overline{B}}^{6.3}$	" " "
					$\underline{\overline{B}}^{6.2}$	" (yellow ")
					$\underline{\overline{B}}^{6.1}$	Endoderm

The first cleavage is bilateral; i.e., it divides the egg, with its presumptive organ regions, into a right blastomere ($\underline{AB^2}$) and a left blastomere ($\overline{AB^2}$). At the second cleavage each of these is

divided into an antero-dorsal blastomere (A^3 and $\overline{A^3}$) and a postero-ventral blastomere (B^3 and $\overline{B^3}$). The third cleavage plane (Fig. 49C, D) separates the smaller cells of the animal hemisphere ($a^{4.2}$, $b^{4.2}$, $\overline{a^{4.2}}$, $\overline{b^{4.2}}$) from the larger cells of the vegetal hemisphere ($A^{4.1}$, $B^{4.1}$, $\overline{A^{4.1}}$, $\overline{B^{4.1}}$).

By the sixth generation (32-cell stage) the organ-forming regions have been segregated into different blastomeres as follows:

Animal hemisphere:

- 14 Ectoderm, epidermis.
- 2 Ectoderm, neural plate.

Vegetal hemisphere:

- 4 Ectoderm and mesoderm, chorda-neural plate.
- 4 Mesoderm, gray crescent.
- 2 Mesoderm, yellow crescent.
- 6 Endoderm cells.

32

The cell lineage of many types of invertebrates has been investigated in a similar manner, and as a result it is now generally recognized that during cleavage the successive generations of blastomeres show a progressive differentiation. Earlier or later, the presumptive organ regions of the fertilized egg are segregated into different groups of blastomeres, each group forming a presumptive organ region of the blastula (page 102).

Later (Chapter VII), experiments will be described which indicate that individual blastomeres may, under different conditions, give rise to parts of the embryo other than those which they produce in the normal course of development.

CLEAVAGE: THE AMPHIOXUS. — In the egg of the amphioxus (Fig. 50), which is isolecithal, cleavage is holoblastic and almost equal. The first cleavage commences as a depression at the animal pole, which later assumes a groove-like form and elongates until it becomes a wide meridional furrow extending around the egg. This constriction deepens until the two hemispheres are completely divided, when each blastomere rounds up into a spherical shape. The second cleavage also commences at the animal pole and is meridional but at right angles to the first, following the second rule of Sachs. The third plane of cleavage

is at right angles to both the first and second and hence would be equatorial if the egg were completely isolecithal. But as the yolk is a little concentrated at the vegetal pole, the nucleus, following Hertwig's first rule, is in the center of the protoplasm, i.e., on the egg axis slightly nearer the animal pole. So the third cleavage plane is nearer the animal pole and accordingly is latitudinal. The quartette of cells in the animal hemisphere is therefore smaller than those in the vegetal hemisphere. The smaller cells are called micromeres; the larger ones, macromeres. The fourth division

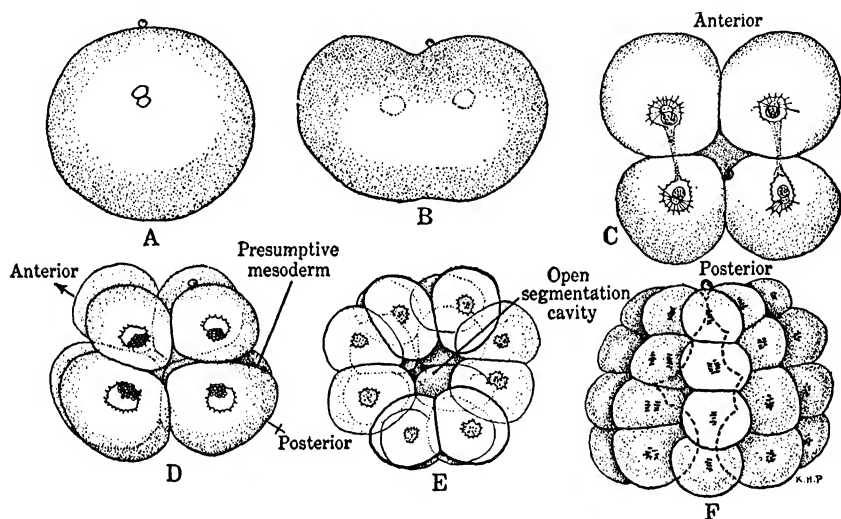


FIG. 50. — Cleavage of the amphioxus egg. A, before cleavage. B, commencing first cleavage, from posterior side. C, second cleavage, from vegetal pole. D, third cleavage, from left side. E, fourth cleavage, from vegetal pole. F, fifth cleavage, side view, segmentation cavity indicated by dotted lines. $\times 166$. (After Conklin, 1932.)

divides each of the eight existing blastomeres in two. There are two planes of cleavage, each meridional, at right angles to the third, and also at right angles to each other. Sometimes the cleavage planes of the fourth division are parallel to each other instead of being at right angles. This makes the bilateral symmetry of the dividing egg quite obvious. In the fifth cleavage 32 cells are produced, again by two planes of cleavage, at right angles to the planes of the fourth, but this time latitudinal and parallel to each other. From this time on cleavage becomes more and more irregular. The early cleavages have been fairly regular;

each has divided the entire egg mass; and the blastomeres, with the exceptions noted, have been almost equal. The blastomeres round up as each cleavage is completed, and a jelly is secreted between them. In this way a small cavity called the segmentation cavity or blastocoel is formed.

Conklin (1933) states that comparison of the cleavage of the amphioxus with that of the tunicates shows a general resemblance between the two in the distribution of the organ-forming substances to the blastomeres, in the generally bilateral type of cleavage, and the order of division; but in all respects the tunicate egg is the more precise and the more precocious in differentiation.

CLEAVAGE: THE FROG. — The frog's egg (Fig. 51) is telolecithal with holoblastic unequal cleavage. Here the first division com-

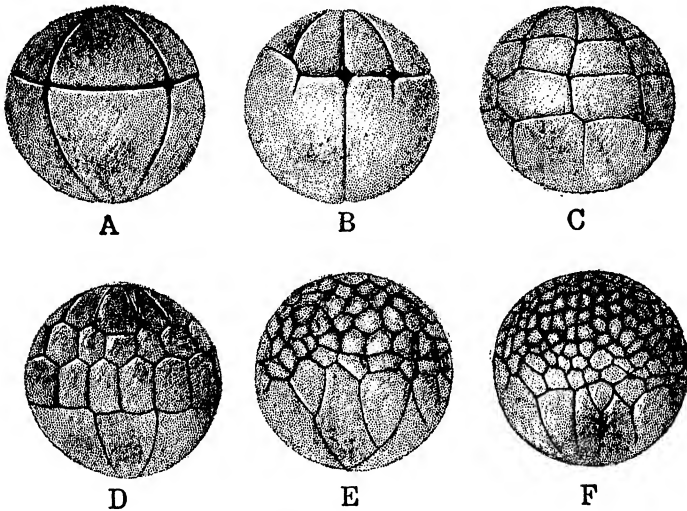


FIG. 51. — Cleavage of the frog's egg. A, third cleavage. B, fourth cleavage (12 cells). C, fifth cleavage. D, sixth cleavage. E, F, later stages. (After Morgan.)

mences as a depression at the animal pole, which elongates and extends around the egg as a shallow furrow until the ends meet at the vegetal pole. The constriction extends inwards and eventually bisects the egg into two blastomeres which round up very slightly. The plane of second division is also meridional and through the animal pole but at right angles to the first. The first two cleavage planes intersect each other at the animal pole; but as the blastomeres round up, the planes no longer form a cross,

but two blastomeres are pushed away from each other, while the other two are in contact forming a short polar furrow between them. The third cleavage is latitudinal, about 20° above the equator, and the micromeres are considerably smaller than the macromeres. Theoretically the fourth and fifth planes of cleavage bear the same relationships to the earlier ones as do those of *Amphioxus*, but actually they are more irregular. The two planes of the fourth cleavage often fail to pass through the vegetal pole and hence become vertical rather than true meridional planes. As these planes originate in the animal hemisphere, the micromeres are divided before the macromeres, so that a 12-cell stage intervenes between the 8-cell and 16-cell stages. Similarly, following Balfour's rule, the latitudinal cleavage plane in the animal hemisphere of the fifth division appears before the corresponding plane in the vegetal hemisphere, so that there is a 24-cell stage before the 32-cell stage is attained.

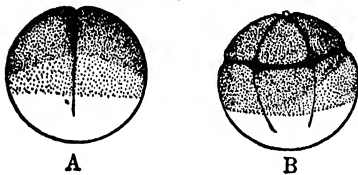


FIG. 52. — The gray crescent of the frog's egg in early cleavage. A, first cleavage, posterior view. B, third cleavage, from left side. $\times 15$, semi-diagrammatic.

The cell lineage of the frog's egg has not been followed in detail as has that of the tunicate or amphioxus. It is known, however, that the first cleavage plane ordinarily divides the gray crescent into two symmetrical halves (Fig. 52A), so that cleavage is normally bilaterally symmetrical from the outset.

The blastomeres receiving the gray crescent material will give rise to notochord and neural plate in later development.

CLEAVAGE: THE CHICK. — In telolecithal eggs with meroblastic cleavage such as that of the fowl, only the protoplasm of the egg, i.e., the blastodisc, is divided, and the cleavage planes do not segment the yolk (Fig. 53). The first furrow commences at the animal pole and extends outwards towards the edges of the blastodisc. The second is formed by two furrows, at right angles to the first, one in each blastomere, which grow towards the first furrow and also towards the edge of the blastodisc. They may join the first furrow at approximately the same point or at separate points, in which case a polar furrow is formed. These four cells are incomplete, as the furrows do not extend all the way to the

yolk nor to the edge of the blastodisc, but remain connected both below and at their margins. From this point on, cleavage is irregular. Some cleavage planes are circular and cut off central cells from marginal. These may be compared with the latitudinal planes of the holoblastic type. Others are radial, like the first

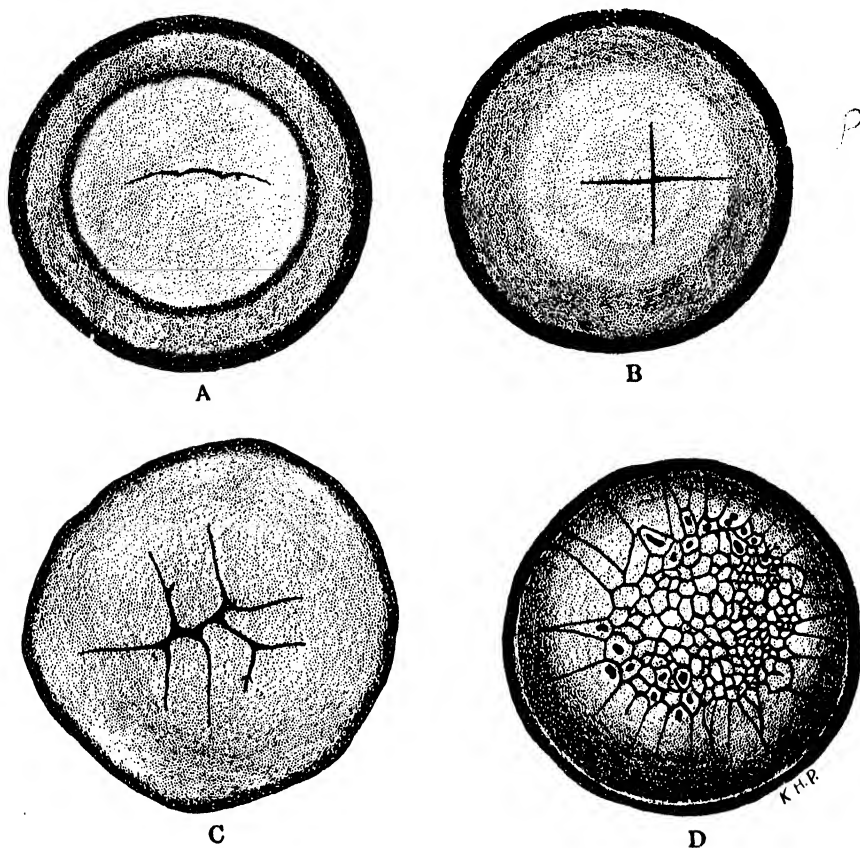


FIG. 53. — Cleavage of the hen's egg. A, first cleavage. B, second cleavage. C, third cleavage. D, later cleavage. All from animal pole. Approx. $\times 12$. (A, B, D, after Kölliker; C, after Patterson.)

and second. Still others are tangential and divide the central cells into upper and lower layers, as in the frog's egg.

CLEAVAGE: MAN AND OTHER MAMMALS. — The cleavage of the human ovum has not yet been observed, but in the egg of the monkey (Fig. 54) and rabbit (Fig. 55) the cleavage is clearly of the equal holoblastic type. In the rabbit the first cleavage takes place

about $22\frac{1}{2}$ hours after coitus. It is equal and complete. The second cleavage follows in about 3 hours. Here the two cleavage spindles frequently lie at right angles to each other so that the four blastomeres assume the form of a cross. Cleavage is now irregular, 5-, 6-, 7-, and 8-cell stages appearing in order. The 8-cell

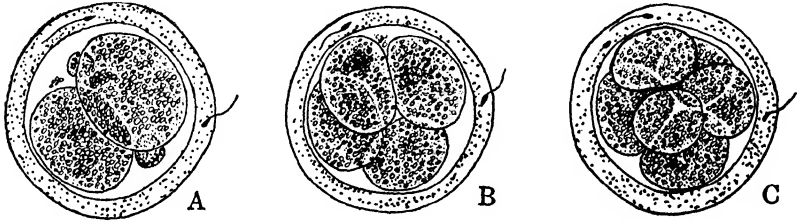


FIG. 54. — Cleavage of the monkey's egg. A, first cleavage. B, second cleavage. C, third cleavage. $\times 170$. (After Lewis and Hartman in Arey.)

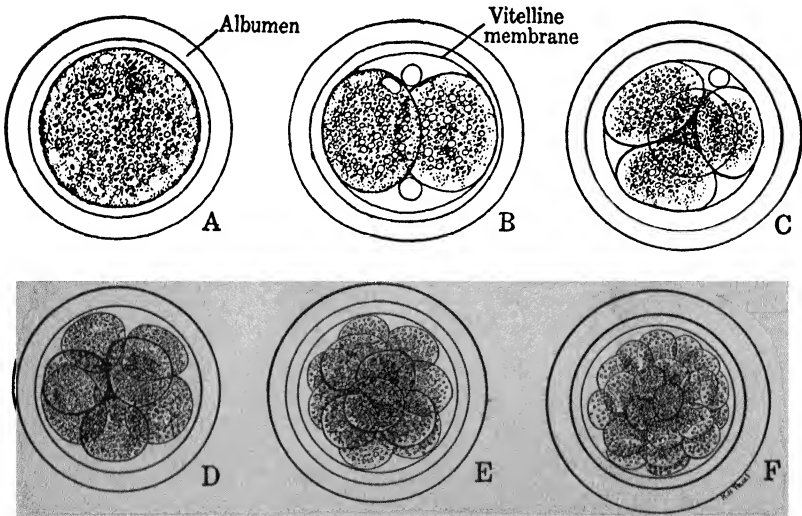


FIG. 55. — Cleavage of the rabbit's egg. A, fertilized egg (note albumen layer). B, first cleavage. C, second cleavage. D, third cleavage. E, fourth cleavage. F, fifth cleavage. $\times 180$. (After Gregory.)

stage is attained about 32 hours after coitus. There is now considerable difference in size, the largest blastomere being almost twice the size of the smallest. The 16-cell stage is reached in another hour and a half. In reaching this stage the cleavage of one blastomere is tangential so that there is always one cell completely enclosed. In later cleavages more tangential cleavages

occur, and this, with the shifting of the blastomeres upon each other, results in a solid mass of cells called a morula.

The blastula. — The period of cleavage terminates in the appearance of the blastula, but this does not mean that cell division comes to an end. The blastula is generally defined as a hollow sphere of blastomeres surrounding a cavity, the blastocoel. But

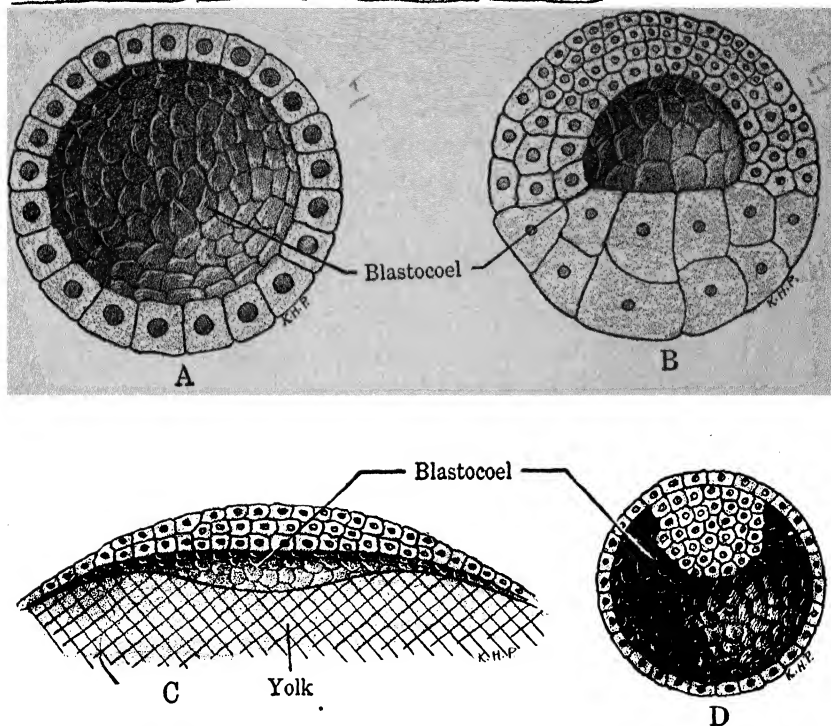


FIG. 56. — Diagrams of vertebrate blastulae. A, coeloblastula following holoblastic equal cleavage (amphioxus). B, coeloblastula following holoblastic unequal cleavage (frog). C, discoblastula following meroblastic cleavage (chick). D, blastocyst (mammals).

this definition does not fit the blastulae formed by meroblastic cleavage. So we shall distinguish three classes of blastulae. The first is of the hollow sphere type (coeloblastula) and is the result of holoblastic equal cleavage (Fig. 56A). A variety of this type, in which the blastocoel is displaced towards the animal pole, is the result of holoblastic unequal cleavage (Fig. 56B).

The second type of blastula (discoblastula) is the result of meroblastic cleavage in which the blastomeres rest in a flat disc,

the blastoderm, on the undivided yolk mass (Fig. 56C). A segmentation cavity later combines with a yolk cavity, formed by the digestion of the yolk underlying the blastoderm, to form a blastocoel. Such a blastocoel is roofed with cells but has a floor of yolk.

The third type of blastula is found only in mammals and is called a blastocyst (Fig. 56D). The solid morula forms a blastocoel which enlarges until it almost separates an outer layer of cells (trophoblast) from an inner cell mass (the embryonic knob).

Presumptive organ regions of the blastula. — As might be inferred from the results of cell-lineage studies, the regions of the blastula will give rise to different parts of the embryo in normal development. In the tunicate and amphioxus, Conklin has mapped out the presumptive organ regions of the blastula, and Vogt and his students, by means of a most ingenious technique, have accomplished the same result for the amphibian blastula. Experimental evidence (Chapter VII) indicates that in the tunicate and amphioxus the organ-forming regions are definitely determined whereas in amphibians, the regions have a greater plasticity and may give rise to parts of the embryo quite different from those formed in normal development.

BLASTULA OF THE AMPHIOXUS. — In the development of the amphioxus we find a good example of the coeloblastula (Fig. 57).

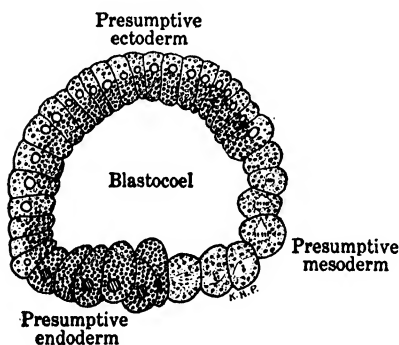


FIG. 57. — Blastula of the amphioxus. Sagittal section. $\times 220$. (After Conklin.)

The blastomeres are arranged in a single layer around the enlarged blastocoel which is entirely cut off from the exterior. The blastomeres at the animal pole are micromeres; those at the vegetal pole are macromeres; the cells at the equatorial belt are transitional in type.

The cells which are to form the mesoderm are rounded and in active mitosis. They are arranged on a crescent on one side of the egg while those which will form the chorda-mesoderm make up a corresponding crescent on the other. The endoderm cells are the larger cells of the vegetal hemisphere.

BLASTULA OF THE FROG. — The blastula of the frog (Fig. 58) resembles that of the amphioxus in all essential characters, but shows minor differences due largely to the greater amount of yolk present. In the first place, the blastoderm is no longer one layer of cells in thickness. Tangential divisions have increased the number of cells so that at the animal pole the blastoderm may be approximately four cells deep. Furthermore, the greater difference in size between the micromeres of the animal pole and the macromeres of the vegetal pole

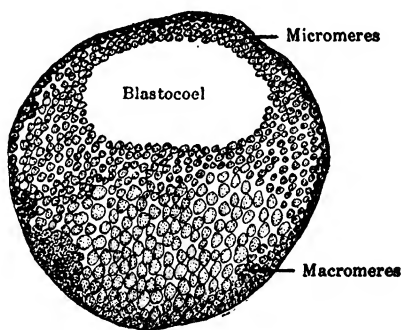


FIG. 58. — Blastula of the frog. Vertical section. (After Brachet.)

result in the blastocoel's occupying an eccentric position entirely within the limits of the animal hemisphere.

The blastula of the frog shows certain regional differentiations. Thus the cells of the animal hemisphere are smaller than those of the vegetal hemisphere. Morgan has pointed out that those arising in the region of the gray crescent are definitely smaller, i.e., dividing more rapidly, than those in any other meridian.

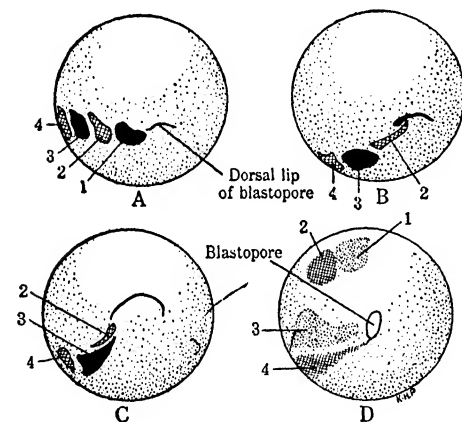


FIG. 59. — Diagrams of the *Triton* egg showing movement of surface areas stained with Nile blue and neutral red during gastrulation. Areas on surface shown with sharp outline, those on interior without outline. (After Vogt.)

Vogt has demonstrated the fate of different regions of the blastula in normal development by marking them with such harmless dyes as Nile blue and neutral red. The stain persists long enough so that the migration of the dyed cell groups can be traced through gastrulation and even later (Fig. 59). He has succeeded in mapping out the surface of the

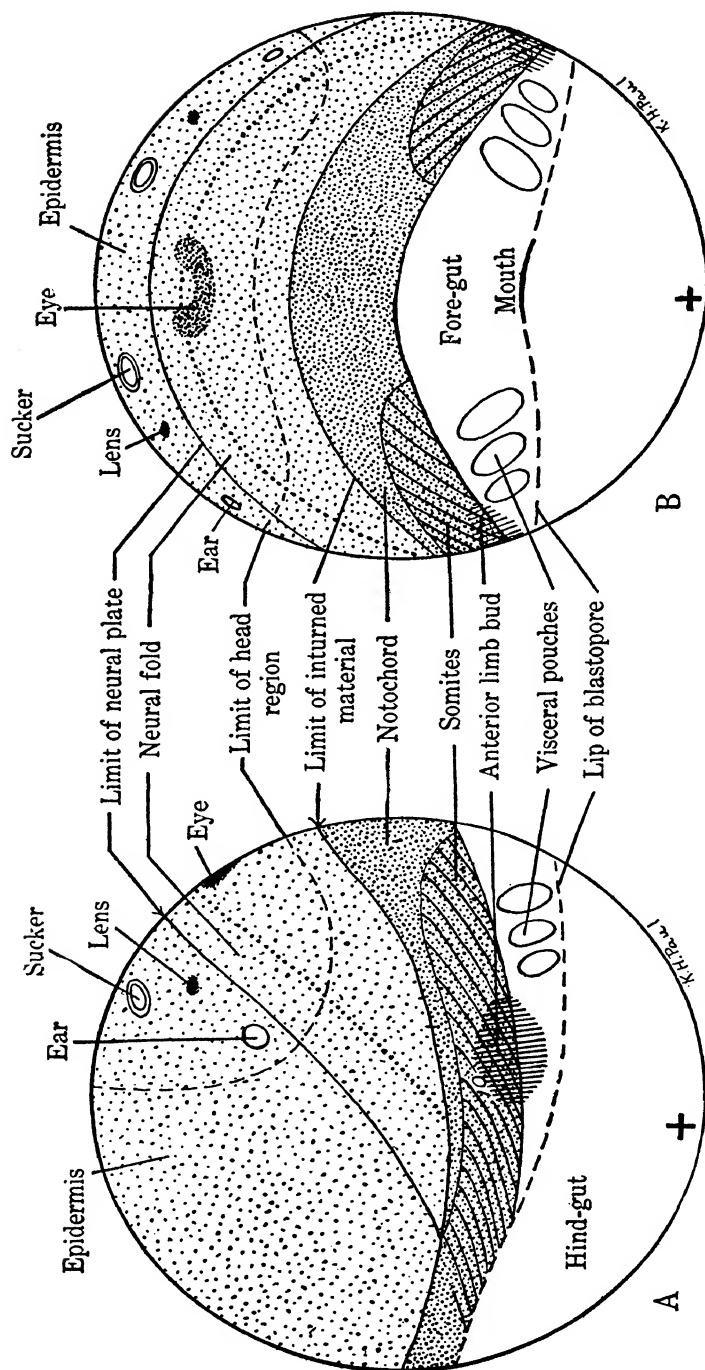


FIG. 60. — Diagrams to show presumptive organ regions of the frog blastula. A, from left side. B, from dorsal surface. The cross indicates the position of the vegetal pole. (After Vogt, 1929.)

blastula into presumptive organ regions, as seen in the diagram (Fig. 60).

BLASTULA OF THE CHICK. — The blastula of the chick is a discoblastula. The blastoderm consists of an inner mass of micromeres completely separated from one another by cleavage planes, and an outer ring of macromeres which are partially separated from one another by incomplete radial cleavage planes only. These latter cells are in direct protoplasmic continuity by means of an outer ring of undivided cytoplasm and a thin lower layer of undivided cytoplasm passing beneath the inner mass (Fig. 61). This undivided cytoplasm is called the periblast. The micromeres of the inner mass are separated from the underlying un-

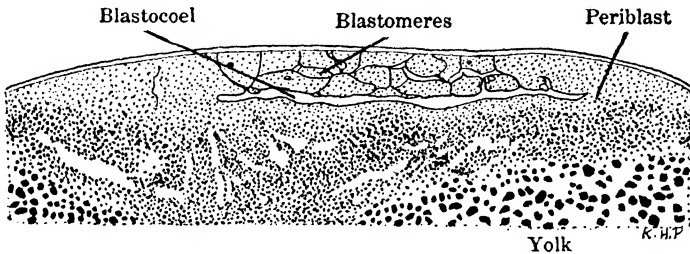


FIG. 61. — Section of early chick blastula. Compare Fig. 53D. (After Patterson.)

divided periblast by means of a thin cleft which is the original blastocoel.

The blastoderm expands over the yolk, new cells being added to the inner cell mass from the outer ring of cells. The periblast, contributing its cytoplasm to the formation of new cells in the outer ring, soon uses up all the material contained in the thin lower layer. Meantime its outer ring, now called the germ wall, expands outward. With the disappearance of the lower layer of periblast, the cells of the inner mass form the roof of a cavity which includes the original blastocoel plus the space originally occupied by the lower layer of periblast. These cells form an area known as the area pellucida because it can be detached from the yolk without carrying any yolk particles and hence appears more transparent. The cells of the outer ring and the germ wall make up the area opaca, so-called because particles of yolk adhere to them when removed from the egg and render them less transparent.

BLASTULA OF MAN AND OTHER MAMMALS.—No human embryo in the blastula stage has been recorded, so a description of the blastocyst of the rabbit will be given in its place. About 75 hours after coitus and while the egg is still in the oviduct, a cleft,

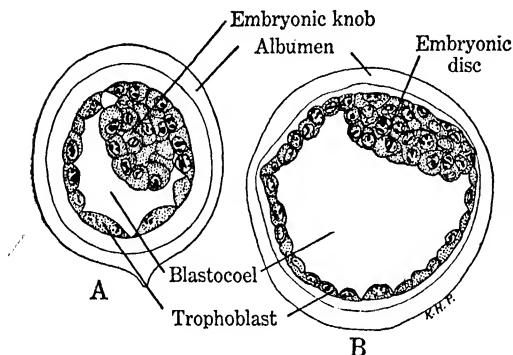


FIG. 62. — Sections of rabbit blastocysts. $\times 200$.
(After Gregory.)

the blastocoel, appears in the morula apparently due to the formation of some fluid. This extends rapidly until an outer layer of cells, the trophoblast, is separated from an inner cell mass, the embryonic knob. The separation is almost complete (Fig. 62A), extending about 270° of the possible 360° . By this time the blastocyst has

reached the uterus and the secretion of fluid is greatly increased, expanding the blastocoel and stretching the trophoblast cells. The embryonic knob flattens against one pole (dorsal) of the trophoblast, and the entire blastocyst increases greatly in size (Fig. 62B). This flattening of the embryonic knob is not characteristic of all mammalian blastocysts.

B. GASTRULATION

The vertebrate blastula becomes converted into a two-layered embryo, or gastrula, through the migration of cells from the exterior to the interior of the embryo. In so doing the blastocoel is obliterated and replaced by a new cavity, the gastrocoel (archenteron), which communicates to the exterior by means of an opening, the blastopore. The cells left on the exterior form the outer germ layer commonly known as ectoderm (ectoblast, epiblast). Those on the inside, lining the gastrocoel, form the inner germ layer, usually called the endoderm (entoderm, entoblast, hypoblast). But, as will be seen later, they may also include cells which will give rise to the middle germ layer, the chordamesoderm, consisting of the mesoderm (mesoblast) and notochord (chorda dorsalis). In such cases the inner layer may be called

mesendoderm (see page 115). The different types of blastulae resulting from different types of cleavage naturally give rise to different types of gastrulae (Fig. 63) according to the means by which the endoderm is segregated from the ectoderm.

Students of gastrulation distinguish five types of cell migrations which will be described briefly here, and developed more fully in later paragraphs.

1. *Invagination* (Fig. 63A). Typical of the coeloblastula resulting from equal holoblastic cleavage. The cells of the animal hemisphere move inward in a con-

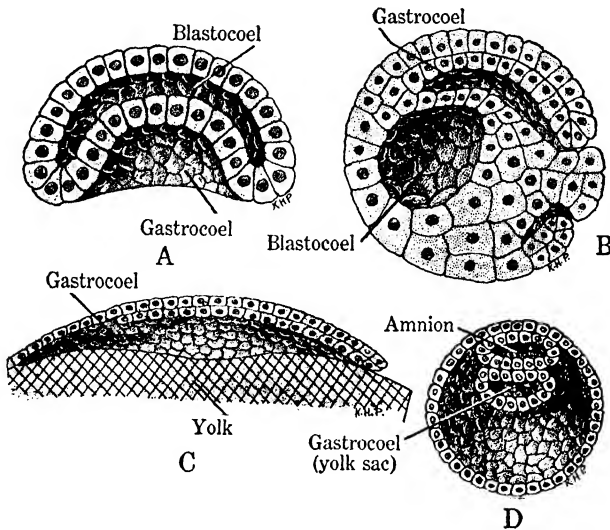


FIG. 63. — Diagrams of vertebrate gastrulation. A, by invagination (amphioxus). B, by epiboly and involution (frog). C, by involution (chick). D, by delamination (mammal).

tinuous sheet, obliterating the blastocoel, until they come to rest against the cells of the animal hemisphere, thus giving rise to a new cavity, the gastrocoel, which opens to the exterior by means of the blastopore. This process is made possible by the continued growth of cells at the lip of the blastopore which roll inward (involution, see 3) as invagination continues.

2. *Epiboly* (Fig. 63B). Typical of the coeloblastula resulting from unequal holoblastic cleavage. The cells of the animal hemisphere grow over the cells of the vegetal hemisphere, creating a gradually narrowing circular fold, the lip of the blastopore. This process also involves the growth and rolling inward of cells at the moving lip (involution, see 3) to form the roof of the gastrocoel.

3. *Involution* (Fig. 63B, C). Typical of the discoblastula resulting from mero-blastic cleavage. The cells at one region of the disc roll inward and spread out under the disc to form the roof of a gastrocoel. The region where involution takes place is the dorsal lip of the blastula. Involution also accompanies invagination and epiboly (see 1 and 2).

4. *Delamination* (Fig. 63D). Typical of the blastocyst in mammals. The lower

cells of the embryonic knob split off as a loose layer which later reorganizes itself to enclose a gastrocoel.

5. *Concrescence* (Fig. 64). As the blastopore narrows, cells which originally lay along the right and left halves of the dorsal lip converge towards each other. And, since the dorsal lip is also growing backward, these cells will form the right and left sides of an axial (antero-posterior) streak.

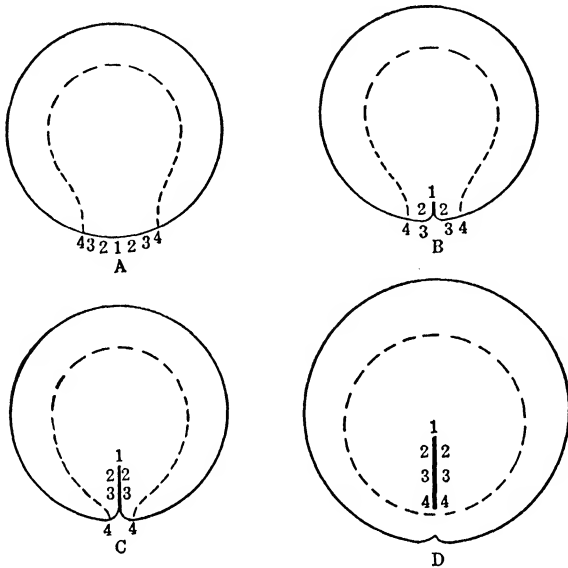


FIG. 64. — Diagrams showing four stages in the process of concrescence. (After Lillie.)

GASTRULATION IN THE AMPHIOXUS. — The first indication of gastrulation is a flattening of the macromeres of the vegetal hemisphere (Fig. 65A). These cells divide less frequently and become more columnar, while the others divide more frequently and become more cubical or spherical in shape. This change in the shape and rate of division, says Conklin (1932), is apparently the principal cause of invagination (Fig. 65B), although it may be due also in part to the resorption of material from the blastocoel jelly, or to exosmosis, for the contents of the blastocoel become less viscous as gastrulation proceeds.

In later stages of gastrulation the gastrula increases in length, owing to the backward growth of the lips of the blastopore (Fig. 65C). While this process is taking place cells are being rolled from the exterior to the interior (involution). The lips of the blastopore grow unevenly, the ventral lip finally turning upward

to reduce the blastopore to a very small opening (Fig. 65D). Conklin expressly denies that this narrowing of the blastopore is caused by the growing together of the right and left halves of the dorsal lip (concrecence). The cells left on the exterior after gastrulation is complete are ectoderm. Those which have been carried to the interior are endoderm, and presumptive chorda-

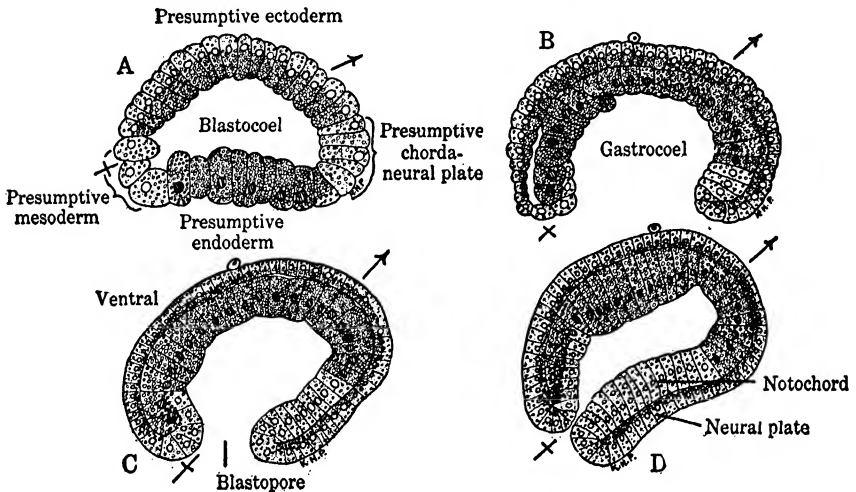


FIG. 65. — Sections of amphioxus embryos during gastrulation. A, blastula (6 hours after fertilization). B, gastrula (9½ hours). C, gastrula (12 hours). D, gastrula (14 hours). Animal pole indicated by presence of polocyte. Antero-posterior axis shown by arrow. All sagittal sections. $\times 180$. (After Conklin, 1932.)

mesoderm. The segregation of the notochord and mesoderm cells is discussed in Section C of this chapter.

In late gastrulation the cells of the ectoderm develop cilia, by means of which the embryo rotates within its fertilization membrane.

GASTRULATION IN THE FROG. — The first stage in the gastrulation of the frog is the formation of a groove on the dorsal side of the embryo in the region of the gray crescent (Fig. 66A). Along this groove, cells are pushed into the interior (involution), while at the same time the cells immediately above the groove are growing down over the surface of the embryo to cover them (epiboly). In this way a two-layered fold is produced, the dorsal lip of the blastopore (Fig. 66D).

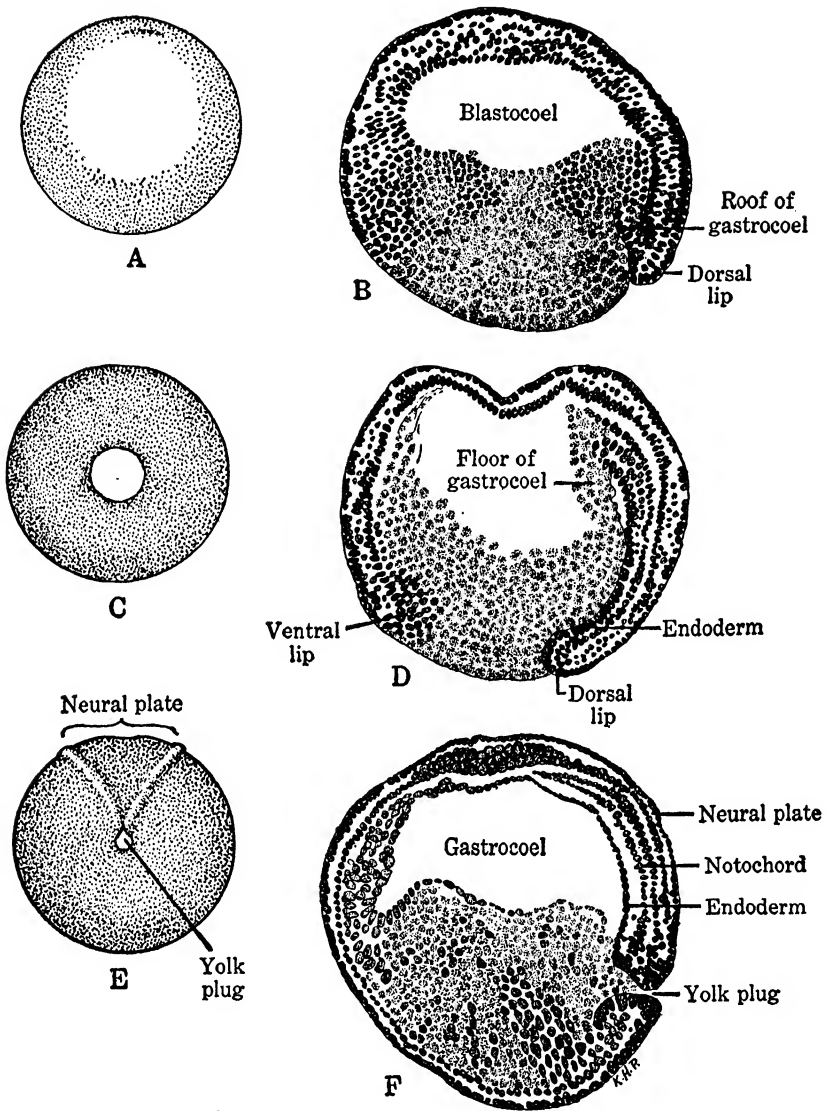


FIG. 66. — Three stages in the gastrulation of the frog's egg. A, dorsal lip stage, from vegetal pole. B, do., sagittal section. C, lateral lip stage, from posterior surface. D, do., sagittal section. E, ventral lip (yolk-plug) stage, from posterior surface. F, do., sagittal section. (B, D, F, after Brachet.)

As the two-layered fold grows down over the cells of the vegetal hemisphere, it extends laterally, thus forming the lateral lips of the blastopore (Fig. 66B). And, since it is covering a spherical surface, the ends of the fold eventually meet to form the ventral lip (Fig. 66C). Epiboly and involution take place at all points on the lip of the blastopore, but chiefly at the dorsal lip, which moves approximately 90° around the egg. At this time the egg presents the appearance of a black sphere with a small white circular area, known as the yolk plug (Fig. 66C).

Within the egg, two distinct phenomena have been taking place. First, the cells turned inward by involution at the dorsal lip have spread out to form the roof of a wide but shallow cavity, the gastrocoel. Second, small cells have arisen from the large yolk-laden cells of the vegetal hemisphere, and these form the floor of the gastrocoel. They join the cells resulting from involution at the anterior end of the gastrocoel (Fig. 66E).

There is now an extensive displacement of the interior cells, resulting from the growth forward of the gastrocoel, and the consequent thinning of its floor. It is still uncertain whether the floor is pushed across the blastocoel, thereby obliterating it, or whether the thin floor is ruptured so that the blastocoel is added to the enlarging gastrocoel (Fig. 66F). In either event the center of gravity in the egg is now altered so that it rotates about a horizontal axis in such a way that the blastopore is carried back to a point a little beyond its starting point, 100° .

The blastopore is now in its definitive position and marks the posterior end of the embryo. The dorsal side, already marked by the appearance of the dorsal lip, is uppermost. In the concluding stages of gastrulation the blastopore narrows to a small slit. This narrowing is brought about by the growing together of the right and left halves of the dorsal lip (concrecence) as epiboly and involution continue.

The cells of the inner layer during later stages of gastrulation appear to be split into two separate layers. The one of these which lines the gastrocoel is endoderm. The other lying between the endoderm and the ectoderm is the chorda-mesoderm. The mode of origin of the latter will be described in the following section.

GASTRULATION IN THE CHICK AND PIGEON. — The blastula of the chick is a disc of blastomeres lying over the undivided yolk.

It is divided into an interior area pellucida and an outer area opaca. This outer area is extending itself in all directions over the undivided yolk (epiboly). The account which follows is based on gastrulation in the pigeon.

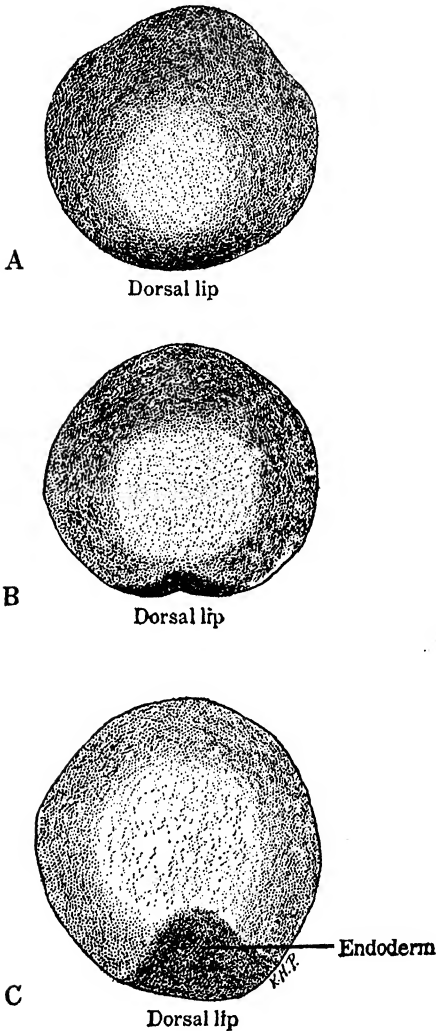


FIG. 67. — Surface views showing three stages in the gastrulation of the hen's egg, from the animal pole. (After Patterson.)

Three zones are distinguishable in the area opaca. First, there is a margin of overgrowth where the cells are completely separated from the yolk. Second comes a zone of junction, whose deeper cells are not separated from the yolk. The third division is the inner zone, whose cells, completely separate from the yolk, are being added to the area pellucida.

The first indication of gastrulation is the thinning of the blastoderm at the posterior end and the complete separation of the cells from the yolk at that region (Fig. 67A). In other words, there is a crescentic area, almost a quarter of the circumference, of the blastoderm which lacks the zone of junction completely. Here the cells roll inward (involution) (Fig. 68) and multiply until they have

spread completely under the upper layer to roof in the old blastocoel and convert it into the new gastrocoel, whose floor is made up of undivided yolk. The slit-like opening where the zone of

junction disappeared is the blastopore, and the rim along which involution took place is the dorsal lip.

There is very little overgrowth at the dorsal lip while involution is taking place, and consequently the edges of the blastoderm on either side swing around to enclose the lip region in the advancing

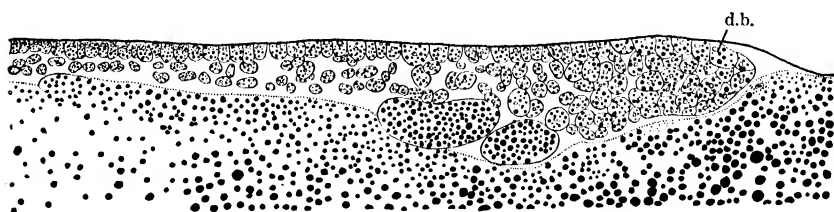


FIG. 68. — Sagittal section through early gastrula of pigeon (36 hours after fertilization). Posterior half of section only. d.b., dorsal lip of blastopore. (From Richards after Patterson.)

germ wall. In this way the blastopore is compressed laterally and concrescence takes place.

GASTRULATION IN MAN AND OTHER MAMMALS.—No human embryo has been observed before the separation of the germ layers. The account which follows is based on the pig. From the lower surface of the embryonic knob, individual cells detach themselves to form a sheet (Fig. 69) which rapidly establishes

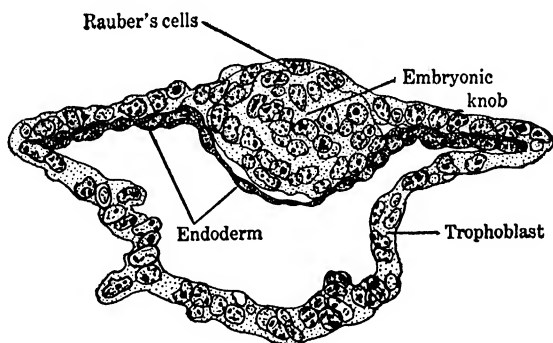


FIG. 69. — Section to show an early stage in the gastrulation of the bat's egg. (After Van Beneden.)

itself as a layer immediately inside the trophoblast, enclosing most of the old blastocoel. We may now consider the trophoblast and the remainder of the embryonic knob as ectoderm and the inner layer as endoderm. The cavity which it encloses is com-

parable to the gastrocoel plus the yolk sac of the egg-laying mammals.

The cells of the trophoblast immediately overlying the embryonic knob (Raubert's cells) now disappear, and the embryonic knob flattens out to become the embryonic disc. This disc lies at the surface and constitutes part of the wall of the blastocyst.

In the primates, judging from studies on the lemur, *Tarsius*, and from the appearance of the earliest human embryo (Fig. 70), the endoderm does not grow out around the entire trophoblast,

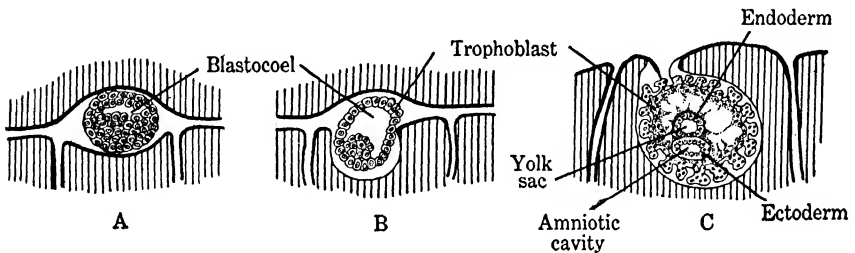


FIG. 70. — Diagrams to show three stages in the gastrulation of the human egg during implantation. The uterine wall indicated by hatching. (Hypothetical based on Teacher; the embryo in C based on Miller.)

but forms a very small vesicle immediately under the embryonic knob. The cavity of this vesicle may be considered a gastrocoel but is more generally known as the "yolk sac."

C. THE MIDDLE GERM LAYER (CHORDA-MESODERM)

During or immediately following gastrulation a third germ layer appears between the ectoderm and endoderm. This layer consists of the notochord (chorda dorsalis), an axial supporting rod found only in the vertebrates and their allies the protochordates, and two sheets of mesoderm on each side of the notochord. Later wandering ameboid cells, originating from the mesoderm and known collectively as the mesenchyme, make their appearance.

The student should note that in many elementary texts the middle germ layer is called the mesoderm and that the notochord is variously derived from mesoderm, endoderm (amphioxus and frog), or ectoderm (chick and mammals). This terminology dates back to the phylogenetic period of embryology (Chapter I), when

it was supposed that a blastula composed of undifferentiated blastomeres gave rise to a gastrula with two separate (primary) layers, and that the mesoderm and the notochord arose separately from one or the other of the so-called primary layers, primitively from the endoderm. Today it is generally recognized that the notochord arises in the same manner and at the same time as the mesoderm. To avoid the clumsy phrase, mesoderm and notochord, many writers are now employing the term chorda-mesoderm for the middle germ layer, and restricting the term mesoderm to the middle germ layer exclusive of the notochord, a usage employed in this text. The compound word mesendoderm (mesentoderm) is now used by many writers to include both the endoderm and the chorda-mesoderm when these layers lie beneath the ectoderm but have not yet segregated from each other.

In collateral reading the student will sometimes encounter the word endo-mesoderm used in connection with mesoderm "originating from" or, better, associated with, endoderm in early development. Similarly the word ecto-mesoderm is employed to designate mesoderm "originating from," or associated with, ectoderm in early development. Other writers use the terms peristomial mesoderm, meaning mesoderm appearing in the region of the blastopore, and gastral mesoderm for mesoderm appearing to arise from the invaginated endoderm. But inasmuch as the middle germ layer can often be traced to definite blastomeres during early cleavage, this distinction is of small importance.

It is well established, however, that among the vertebrates the movement of the presumptive chorda-mesoderm to its definitive position in the roof of the gastrocoel is intimately associated with the formation and closure of the blastopore. This is true no matter whether the blastopore is a large circular opening as in the amphioxus and the frog, or reduced to a primitive streak by concrescence as in the chick and man.

The later history of the germ layers. — With the segregation of the three germ layers, the presumptive organ regions are now located in one or another of the three. But it must not be supposed that the organs of the adult are exclusively ectodermal, endodermal, or mesodermal. On the contrary, most of them contain material from at least two, and sometimes all three. In

Part III will be found an account of the development of the different organ systems, classified according to the germ layer from which arise the tissues associated with their special functions. Meantime the following table is presented.

TABLE 7
DERIVATIVES OF GERM LAYERS

Ectoderm	Chorda-mesoderm	Endoderm
	A Notochord B Mesoderm	
1. Epidermis of skin and all openings into the body 2. Epithelia of eye, ear, and nose 3. Nervous system, including interrenal glands, pituitary gland (in part), pineal gland 4. Epithelium of amnion and chorion	1. Epithelium of coelom and exocoel 2. Nephric (excretory) system 3. Genital (reproductive) system 4. Suprarenal gland 5. Blood-vascular system 6. Connective tissue including skeleton 7. Musculature 8. Dermis of skin	1. Epithelia of digestive tube, including thymus gland, thyroid gland, parathyroid gland, internal respiratory organs, yolk sac, and allantois

THE MIDDLE GERM LAYER IN THE AMPHIOXUS. — As mentioned in earlier sections, Conklin (1933) has been able to distinguish the mesoderm cells in the amphioxus in the blastula stage (Fig. 57), where they form a crescent of small rounded blastomeres in the region where the ventral and lateral lips of the blastopore will form. The notochord cells, associated with those which will later give rise to the neural plate, occupy a corresponding chorda-neural crescent at the dorsal lip. After the invagination of the endoderm the cells of the mesoderm and notochord form the lip of the blastopore, the notochord cells at the dorsal lip, mesoderm at the ventral and lateral lips. As the lips of the blastopore grow backward, these cells are carried to the interior by involution (Fig. 65).

When the ventral lip grows upward, the mesodermal crescent is tilted up behind so that its arms run in an antero-posterior direction to form the angles between the roof and sides of the gastrocoel (Fig. 71). In the meantime the notochord cells, also carried into the interior, form a flat plate between the two arms of the mesoderm. Thus the roof of the gastrocoel is composed

of three strips of chorda-mesoderm, mesoderm on each side, notochord in the middle.

A longitudinal groove in the notochord plate deepens, and the folds on either side come together to form a solid cord separate

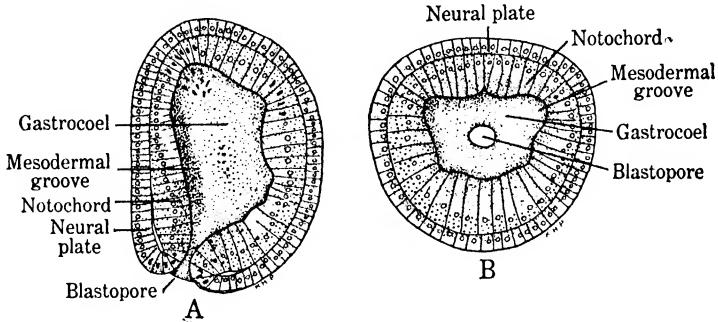


FIG. 71. — Optical hemi-sections of amphioxus gastrula (14 hours after fertilization). A, left inside. B, posterior to show notochord and mesodermal groove inside. $\times 166$. (After Conklin, 1932.)

from the ectoderm above and the mesoderm on either side. The mesodermal grooves (Fig. 72) also become deeper. Transverse constrictions meantime appear in the lateral grooves, which

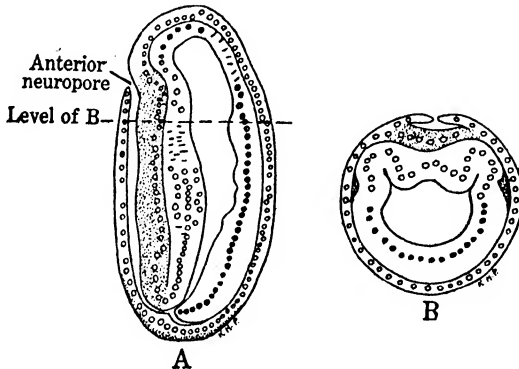


FIG. 72. — Sections of amphioxus embryo (19 hours after fertilization). A, sagittal section. B, transverse section. $\times 166$. (After Conklin.)

divide them into a series of pouches (enterocoels). Finally these pouches are constricted off from the gastrocoel and become the paired somites (Fig. 73).

The endoderm, which formerly occupied the floor and anterior end of the gastrocoel, extends to form new sides and a new roof.

The gastrocoel, now for the first time completely lined with endoderm, is the primordium of the digestive tube.

The cells of the chorda-neural crescent remaining on the exterior of the embryo give rise to the neural plate on the dorsal surface. They are covered by the ventral lip of the gastrula as it grows over the dorsal side of the embryo. Beneath this covering

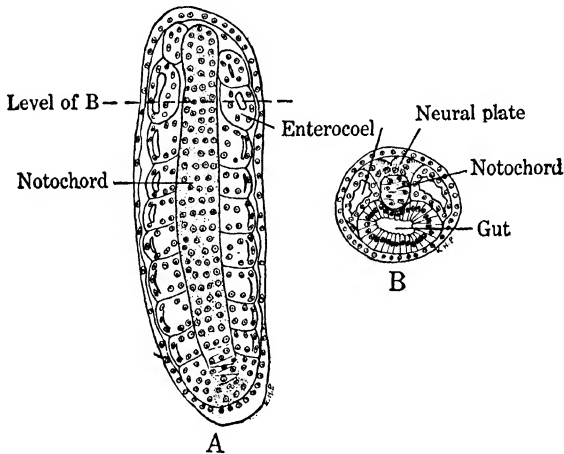


FIG. 73. — Sections of amphioxus larva ($24\frac{1}{2}$ hours after fertilization). A, frontal section. B, transverse section. $\times 166$. (After Conklin.)

there appears a longitudinal groove with a fold on either side. These folds arch up and meet in the ventral line to form the neural tube.

THE MIDDLE GERM LAYER IN THE FROG. — As noted in earlier sections, we owe to Vogt (1929) the identification of the various regions on the amphibian blastula. This identification was accomplished by staining small regions of the blastula surface with harmless dyes and tracing their movements during and after gastrulation (Fig. 59). He finds that the material first to be turned in at the dorsal lip is endoderm. Immediately anterior and dorsal to this is a crescent-shaped area which will give rise to the notochord. On either side of this are the horns of a crescent extending from the other side of the blastula which will become mesoderm. Immediately anterior to the chorda crescent is the crescent-shaped area of the neural plate, the two together being equivalent to the chorda-neural crescent of the amphioxus. The

mesodermal crescent also corresponds to the mesodermal crescent of the amphioxus except that its arms already extend dorsally.

In the gastrulation of the tailed amphibia (urodeles), the material turned in at the dorsal lip is notochord and mesoderm, so that the roof of the gastrocoel is chorda-mesoderm as it is in the amphioxus, and endoderm cells must grow up from the sides and floor to form a new roof.

In the frog, however, the first material to roll in at the dorsal lip of the blastopore is endoderm and notochord (Fig. 60). When the material from the mesodermal crescent rolls in, instead of following the endoderm, it wedges in between the endoderm and ectoderm (Fig. 66F), so giving the appearance of splitting off from the endoderm in the roof of the gastrocoel. The roof and sides of the gastrocoel are, therefore, endodermal except for a narrow dorsal strip represented by the notochord (and a narrow strip beneath it, the hypochord). When the notochord (and hypochord) separate from the roof, this small gap is closed by endoderm and the roof is completely endodermal.

As the endoderm, notochord, and mesodermal regions are turned in around the lips of the blastopore the overgrowth of the lips covers the large yolk-laden cells from which the floor of the gastrocoel is produced. Meantime the expanding cells from the ectodermal region of the blastula occupy the region formerly held by the material which has been turned in. Now the

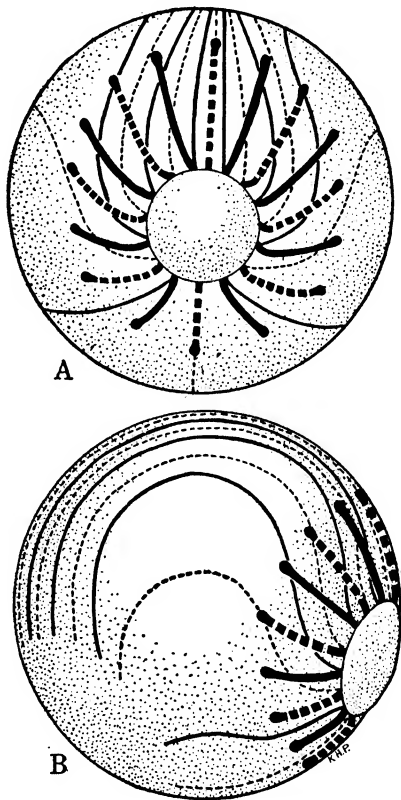


FIG. 74. — Diagrams showing direction of displacements during amphibian gastrulation. A, from posterior surface. B, from left side. Thick lines on exterior surface. Thin lines on interior. (After Vogt, 1929.)

dorsal lip of the blastopore is the one at which epiboly and involution take place most rapidly. Consequently materials on the right and left of the mid-dorsal region are stretched towards the medial line to take the place of the material lost by involution (Fig. 74). In this way the two arms of the mesodermal crescent move together to form parallel strips on either side of the noto-

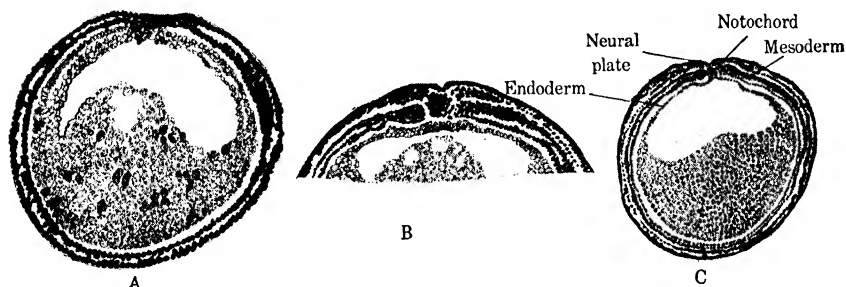


FIG. 75. — Transverse sections to show three stages in the origin of the notochord and mesoderm in the frog embryo. (After Brachet.)

chord. Similarly the two horns of the neural crescent move together to form parallel strips which eventually enclose the blastopore at the posterior end, while the neural plate itself occupies a longitudinal dorsal position on the gastrula. All the rest of the surface is now material which will form the epidermis of the skin.

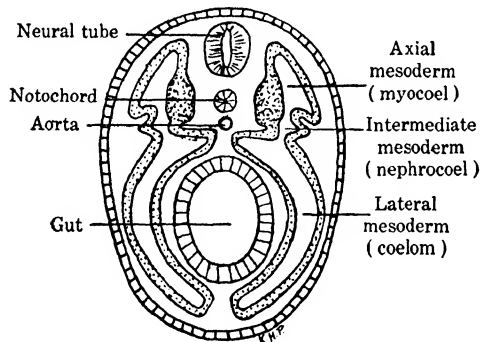


FIG. 76. — Diagram of a transverse section of vertebrate embryo to show the regions of the mesoderm and coelom.

The mesoderm continues its growth between the ectoderm and endoderm (Fig. 75) until it forms a continuous sheet except at the blastopore. The material on either side of the notochord is separated by transverse constrictions into blocks or somites, corresponding to the somites

of the amphioxus. Next comes an intermediate zone from which the gonad and kidney will arise. The remainder splits into an outside (somatic) layer closely applied to the ectoderm, and an

inner (splanchnic) layer similarly applied to the endoderm. The space between (Fig. 76) is the coelom.

The neural plate develops a longitudinal groove, surrounded at the anterior end and sides by ridges known as the neural folds. The embryo has now reached the stage known as the neurula (Fig. 112).

THE MIDDLE GERM LAYER IN THE CHICK.— Mesoderm formation in the chick takes place after the egg has been laid and incubation begun. At about the sixteenth hour (Patten) the blastoderm is considerably lengthened in an antero-posterior direction, and has an axial thickening known as the primitive streak (Fig. 77). This streak represents the dorsal lip of the blastopore laterally compressed through concrescence as explained on page 108. The germ wall has grown together behind the primitive streak and is advancing out over the yolk. In a more advanced embryo the primitive streak is differentiated into a primitive groove in the middle, primitive folds on either sides, a primitive pit at the

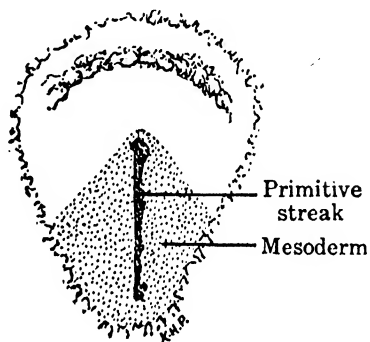


FIG. 77. — Blastoderm of the chick at 15 hours of incubation. (After Duval.)

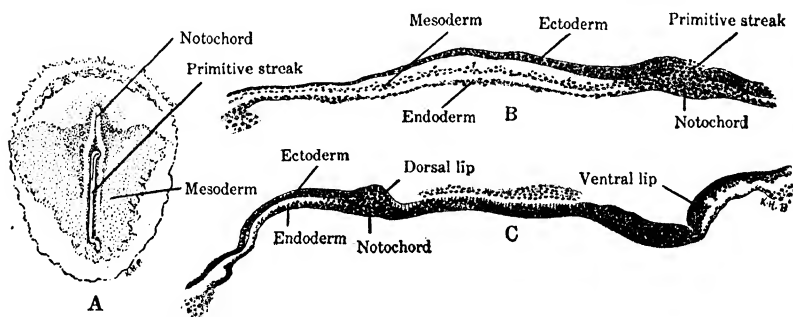


FIG. 78. — Blastoderm of chick to show early stage in development of notochord. A, surface view at 20 hours (after Duval). B, transverse section, left half only. C, sagittal section. (B, C, after Lillie.)

anterior end of the groove, and a primitive (Hensen's) node in front of the pit where the primitive folds unite (Fig. 78).

Sections reveal that from the sides and posterior end of the primitive groove, cells are growing outward, between the ectoderm and the endoderm, to form a sheet of mesoderm. At the anterior end a narrow strip of cells grows forward to form the notochord.

During the remainder of the first day of incubation the area pellucida increases in length, particularly in the region directly in front of the primitive streak. This appears to displace the primitive streak rearwards, and during this time the streak actually shortens.

The mesoderm growing out to the sides is carried forward in this movement and so comes to lie close to the advancing notochord. Furthermore, two horns of mesoderm grow forward, later to curve in and meet in front of an area which contains ectoderm and endoderm only (proamniot). The mesoderm on either side of the notochord thickens to form a segmental zone, so called because it will shortly be divided by transverse constriction into somites, exactly as in the frog. Six pairs of somites are present at the end of the first day (Duval). There is a zone of intermediate mesoderm. The remaining or lateral mesoderm, growing out into the area opaca, splits tangentially into an outer somatic and an inner splanchnic layer, as in the frog. In the splanchnic mesoderm, thickenings appear in the inner region of the area opaca. They mark the primordium of the area vasculosa (Fig. 79).

The ectoderm and endoderm of the clear area give rise to a crescentic fold at the anterior end which is called the head fold as it is the primordium of the head of the embryo. It contains a pocket of endoderm known as the fore-gut, distinguished by the possession of a cellular floor. There is an opening known as the anterior intestinal portal between the fore-gut and the mid-gut, whose floor is the undivided yolk.

The ectoderm in front and to either side of the notochord is the neural plate. It develops a groove and folds shortly before the end of the first day, and at 24 hours of incubation the folds have met in the region of the brain to form a tube but have not as yet fused together.

THE MIDDLE GERM LAYER IN MAN. — The earliest human embryo is the "Miller" ovum (Fig. 69). This specimen, supposed

to be about 13-14 days old, consists of an outer vesicle, the trophoblast, containing two smaller vesicles, one of which, lined with endoderm, represents a small gastrocoel (yolk sac) the

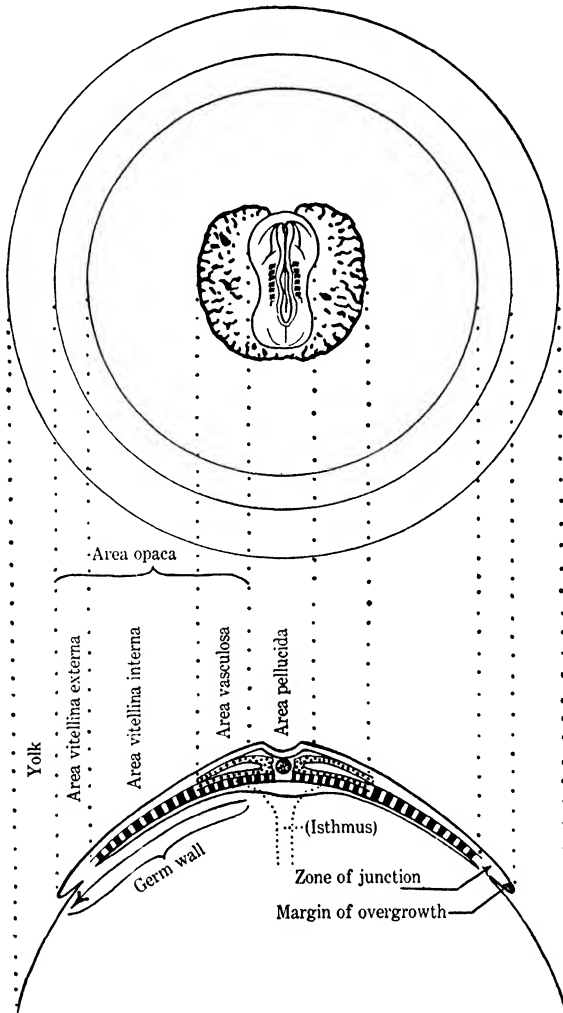


FIG. 79. — Diagram showing embryonic and extra-embryonic areas of chick embryo at 24 hours of incubation. Above, surface view; below, transverse section.

other of ectoderm surrounds a cavity (the amnion, Chapter V). Where the two vesicles are in contact a circular disc of ectoderm and endoderm pressed together represents the embryonic disc. In later specimens this embryonic disc develops a primitive streak,

quite as in the chick blastoderm (Fig. 80). Notochord and mesoderm develop in much the same way, somites appearing at the end of the first month. A head fold and neural groove appear in similar fashion.

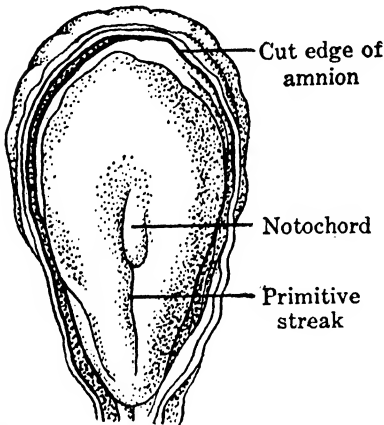


FIG. 80. — Surface view of embryonic disc in human embryo after amnion has been cut away. $\times 40$. (After Heuser.)

SUMMARY

During cleavage the fertilized egg is divided into a large number of daughter cells or blastomeres which arrange themselves about a cavity to form the blastula. The pattern of cleavage and the form of the blastula vary according to the amount and distribution of the yolk in the fertilized egg. The presumptive organ regions of the fertilized egg are segregated into different groups of cells which

compose the presumptive organ regions of the blastula.

During gastrulation, the blastomeres are reorganized into different strata or germ layers about a new cavity, thus forming a gastrula. The method of gastrulation varies according to the type of blastula formed after cleavage. The two layers segregated during gastrulation are usually known as the ectoderm and endoderm, but it must be recognized that one or the other of these so-called primary layers includes the presumptive mesoderm as well.

In the concluding period of germ-layer formation, the middle germ layer or chorda-mesoderm, including the notochord and the mesoderm proper, is segregated from the other germ layers to occupy a middle position between them.

While the germ layers are being segregated from each other the primordia of certain organs are arising from their respective presumptive regions. Thus the notochord is separated from the mesoderm proper, the neural plate from the presumptive epidermis. In the mesoderm proper, the somites begin to take form, and the somatic layer separates from the splanchnic to form the coelom.

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CHAPTER VI

EMBRYONIC FORM AND EXTRA-EMBRYONIC STRUCTURES

After the germ layers have been segregated, the primordia of several great organ systems are already localized. Before proceeding to an account of the way in which the organ systems develop from the different germ layers (organogeny), we must examine the way in which the vertebrate body assumes its form. This is found to be closely connected with certain structures (adnexa) which develop also from the germ layers and play an important part in embryonic (and fetal) life, but which are discarded before hatching (or birth). These extra-embryonic structures are the yolk sac, the amnion, chorion, and allantois, as well as a structure found only in the mammals, the placenta.

A. THE FORM OF THE BODY

The general form of the vertebrate body is cylindrical, while the form of the vertebrate egg is spherical. There are in general

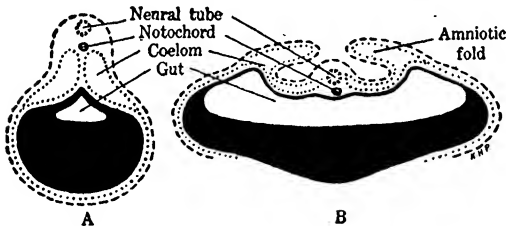


FIG. 81. — Diagrammatic transverse sections showing effects of yolk on form of embryo. A, small yolked embryo (frog). B, large yolked embryo (chick). (After Assheton.)

two methods of growth by means of which the cylindrical shape is attained. In the first, characteristic of small-yolked eggs with a spherical gastrula, the main factor is growth in length, along the antero-posterior (cephalo-caudal) axis. In the second

type, which is characteristic of large-yolked eggs, the embryo is modeled from a flat disc into the form of a cylinder connected with a great yolk sac by some sort of pedestal or stalk. Much of this modeling is done by the outgrowth of the head and the tail respectively, especially among the anamniote vertebrates, but there

is also some actual undercutting, especially evident among the Amniota. This undercutting is accompanied by the formation of amniotic folds, as will be seen in the development of the chick. A diagram of cross-sections through the body of a small-yolked embryo (Fig. 81A) and a large-yolked embryo (Fig. 81B) will make clear the difference between the cylindrical embryo and the plate-like embryo before it has been remoulded. In the amniote vertebrates with a large-yolked egg the embryo develops from

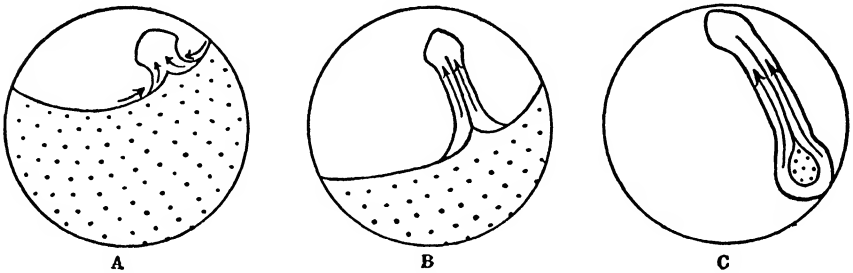


Fig. 82. — Diagrams to show growth in length by conrescence. Arrows indicate direction of growth. (After Assheton.)

material at the edge of the blastoderm, and as this is rolled together in conrescence the embryo increases in length (Fig. 82).

✓ **General plan of the body.** — The body of the vertebrate is basically a tube within a tube, i.e., a digestive tube within a body tube (Fig. 76).

The digestive tube is endodermal in origin and originates from the gastrocoel. Here again the small-yolked form has a tubular intestine from the beginning. It is only necessary to form anterior and posterior openings, for the blastopore either closes or is roofed in by the neural folds. The new openings arise from ectodermal pits, the stomodæum at the anterior end, the proctodæum at the posterior end. In general these openings are not completed until after the yolk has been wholly consumed. The gastrocoel of large-yolked embryos has only a roof and sides of endoderm, for the floor is composed of the yolk. Hence the rolling in or undercutting of the body commencing at the head end, and later at the tail end, forms a pocket at each end, the fore-gut and hind-gut respectively. The mid-gut is the remainder of the open gastrocoel connected with the developing yolk sac by means of the yolk stalk.

Between the two tubes lies the mesoderm. The ventral mesoderm of small-yolked embryos (lateral of large-yolked forms) splits into a somatic and splanchnic layer. The first of these is closely applied to the ectoderm to form the somatopleure; the second is associated with the endoderm to form the splanchnopleure. The space between is the coelom or body cavity. Other and lesser antero-posterior tubes such as the neural tube, formed from ectoderm, and axial blood vessels, e.g., the aorta, formed from mesoderm, are indicated in the figure and will be discussed in later chapters.

Metamerism. — With growth in length is associated a second factor in the development of the vertebrate body, that of metamerism. This is first indicated by the appearance of metameres

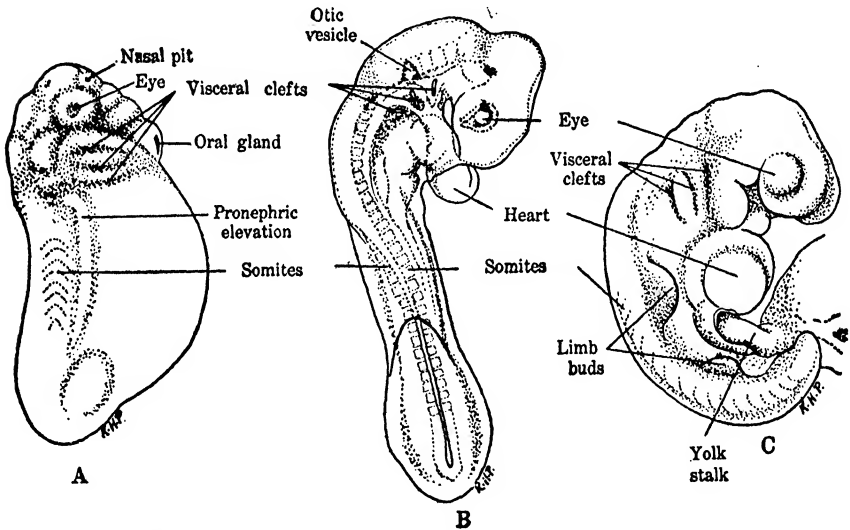


FIG. 83. — Diagrams of early embryos to show similarities in body form. A, frog (after W. Patten). B, chick (after Kerr). C, man (after His).

such as the enterocoels in the amphioxus or somites in the true vertebrates. In later organogeny are found further evidences of metamerism in the nervous system, nephric system, vascular system, and others. However, the primary metamerism of the body is shown in the mesoderm. The somites are formed successively, commencing at the anterior end and therefore affording a basis of classifying the early embryos of any species by the number of these units present (Fig. 83).

The head. — The vertebrate body is distinguished by a well-marked region at the anterior end, containing the mouth, visceral arches, special sense organs (nose, eye, and ear) and the highly developed brain. Herein the amphioxus differs from the vertebrates, for it has so little head that some zoölogists make a special group (Acraniata) to contain it.

The anterior end of the body is already determined in the vertebrate egg (animal pole). It is the surface opposite that of the blastopore, or in front of the primitive streak. It is the region where the neural folds first arise and where they first meet. It is the first part of the body to be freed of the yolk in the large-yolked embryos. A glance at the diagrams of early embryos (Fig. 83) will suffice to prove that this is the most highly differentiated part of the body.

In the Amniota the head is inclined ventrally at the region of the branchial arches. This cervical flexure causes a constriction (Fig. 83) which is the primordium of the neck, a region found only in reptiles, birds, and mammals.

The tail. — All vertebrate embryos, even those of species in which the adult is tailless (frog, man), develop a well-marked tail in early development. This region is characterized by the absence of a digestive tube and coelom. It develops early in the anamniotes, where it is of great use to the free-swimming larva, but more slowly in the amniotes.

The appendages. — The paired appendages of vertebrates arise as buds (Fig. 83C) which later develop into fins or limbs. Limb buds do not appear in the amphioxus or the cyclostomes. In all other vertebrates which do not possess paired appendages in the adult condition, it is said that limb buds appear in the embryonic life and are resorbed later.

BODY FORM OF THE FROG. — The spherical egg of the frog, being only moderately telolecithal, is converted into the cylindrical shape of the embryo principally through the growth of the head and of the tail.

In the head region the neural plate is much wider than elsewhere, and when the neural folds close in to form the neural tube the brain will be larger than the spinal cord. On either side of the head the optic vesicles, the primordia of the eyes, push out from the brain and make well-marked bulges. The ectoderm im-

mediately external to each optic cup will later give rise to the lens of the eye. Anterior to each eye is a depression in the ectoderm, the nasal (olfactory) pit. These pits are the primordia of the nose. Posterior to each eye a similar otic (auditory, acoustic) pit originates, the primordium of the inner ear. On the ventral side, folds of ectoderm give rise to the ventral sucker (mucous gland) in the form of the letter V. Between the limbs of the V there appears an ectodermal pit called the stomodeum or primordium of the mouth. On the ventral side of the body, just ante-

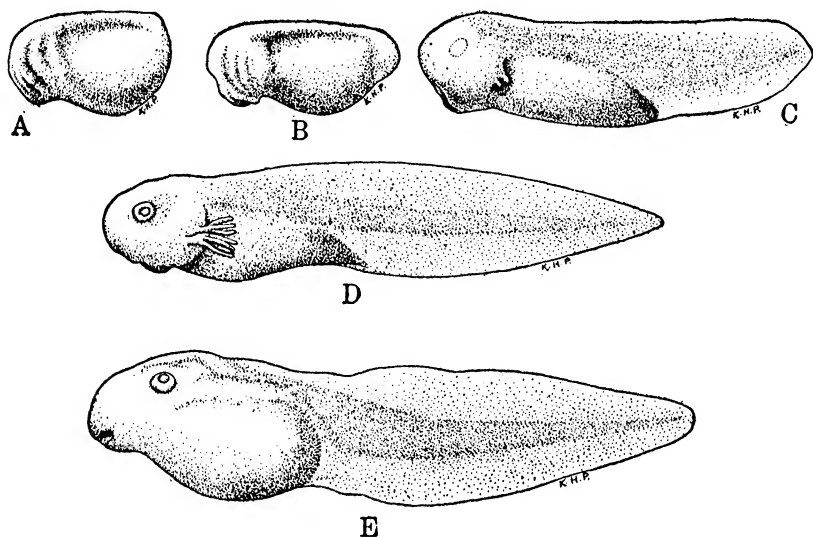


FIG. 84. — Growth of the frog embryo. A, late neurula, 2.4 mm. B, embryo of 3 mm. C, embryo of 6 mm., just hatched. D, young larva, external gill stage, 9 mm. E, larva, internal gill stage, 11 mm. (Measured alive and drawn after preservation. $\times 10$.)

rior to the base of the tail, a similar pit, the proctodeum, is the primordium of the cloacal opening.

On the sides of the head five dorso-ventral grooves appear (in the order I, V, II, III, IV). These are the visceral (branchial, "gill") grooves, some of which will later break through into corresponding outpushings from the fore-gut, the visceral (pharyngeal, "gill") pouches, to form the visceral (pharyngeal, "gill") clefts. For the present we need simply note that they separate six transverse bars or ridges which are known as the visceral

arches. Each visceral arch contains an aortic arch. (See Table 8.) Arch I (mandibular) contributes to the formation of the jaws. Arch II (hyoid) contributes to the gill cover (operculum) and to the support of the tongue. Arches III, IV, and V are often known as branchials 1, 2, and 3, respectively. On arches III, IV, and V develop outgrowths which become the external gills

TABLE 8
PHARYNGEAL DERIVATIVES

Pouches (<i>From endoderm</i>)	Arches	Clefts	Aortic Arches	Grooves (<i>From ectoderm</i>)
	Visceral arch I (mandibular)		Aortic arch I	
Visceral pouch I (hyomandibular)		Visceral cleft I (spiracle of elasmobranchs)		Visceral groove I
	Visceral arch II (hyoid)		Aortic arch II	
Visceral pouch II		Visceral cleft II		Visceral groove II
	Visceral arch III (1st branchial)		Aortic arch III	
Visceral pouch III		Visceral cleft III		Visceral groove III
	Visceral arch IV (2nd branchial)		Aortic arch IV	
Visceral pouch IV		Visceral cleft IV		Visceral groove IV
	Visceral arch V (3rd branchial)		Aortic arch V	
Visceral pouch V		Visceral cleft V		Visceral groove V
	Visceral arch VI (4th branchial)		Aortic arch VI	
Visceral pouch VI (vestigial in frog)		Visceral cleft VI (lacking in frog)		Visceral groove VI (lacking in frog)

(branchiae). That on V is rudimentary. Later a fold grows from arch II to cover the external gills completely on the right, but with an opening on the left known as the atriopore ("spiracle"). While this is taking place the grooves between arches II, III, IV, V, and VI break through into the corresponding visceral pouches to form the visceral clefts. Internal gills (demi-branchs) develop in the clefts, and the external gills disappear. Meantime the mouth has opened and developed horny jaws.

The tail arises by the backward growth of the tissue in the neural folds (Bijtel) at the point where they united over the blastopore. The notochord and neural tube grow backward, carrying epidermis and muscle-forming material with them. Dorsal and ventral folds make the tail fin.

The paired limbs arise as limb buds. The anterior buds arise first but are concealed beneath the operculum. The one on the left side appears first, pushing through the atriopore.

BODY FORM IN THE CHICK. — The body of the chick is cut off from the blastoderm by the outgrowth of a head fold accompanied by an undercut, the subcephalic pocket, which appears during the first day of incubation. This fold extends backward in the form of an inverted U as the lateral folds arise. These are also accompanied by undercuts known as the lateral sulci. Finally there is a posterior tail fold accompanied by a subcaudal pocket appearing on the third day. Outgrowth at the folds with some undercutting as well causes the body of the embryo to stand up from the surrounding blastoderm to which it is attached by a short pedestal, the umbilical stalk. The head bends down sharply at the cephalic flexure, but pressing against the yolk, it turns or twists toward the right so that the left side of the head rests on the yolk. The ventral bend is known as flexure, the dextral twist is known as torsion. Flexure and torsion commence in the middle of the second day of incubation, and continue in a caudal direction until, at the end of the fourth day, the chick lies completely on its left side.

The primordia of the brain and sense organs arise much as they do in the frog. A stomodeum appears early in the third day of incubation, the proctodeum during the fourth day. Four visceral grooves (in the order I, II, III, IV) and five arches appear between the end of the second and beginning of the fourth day of incuba-

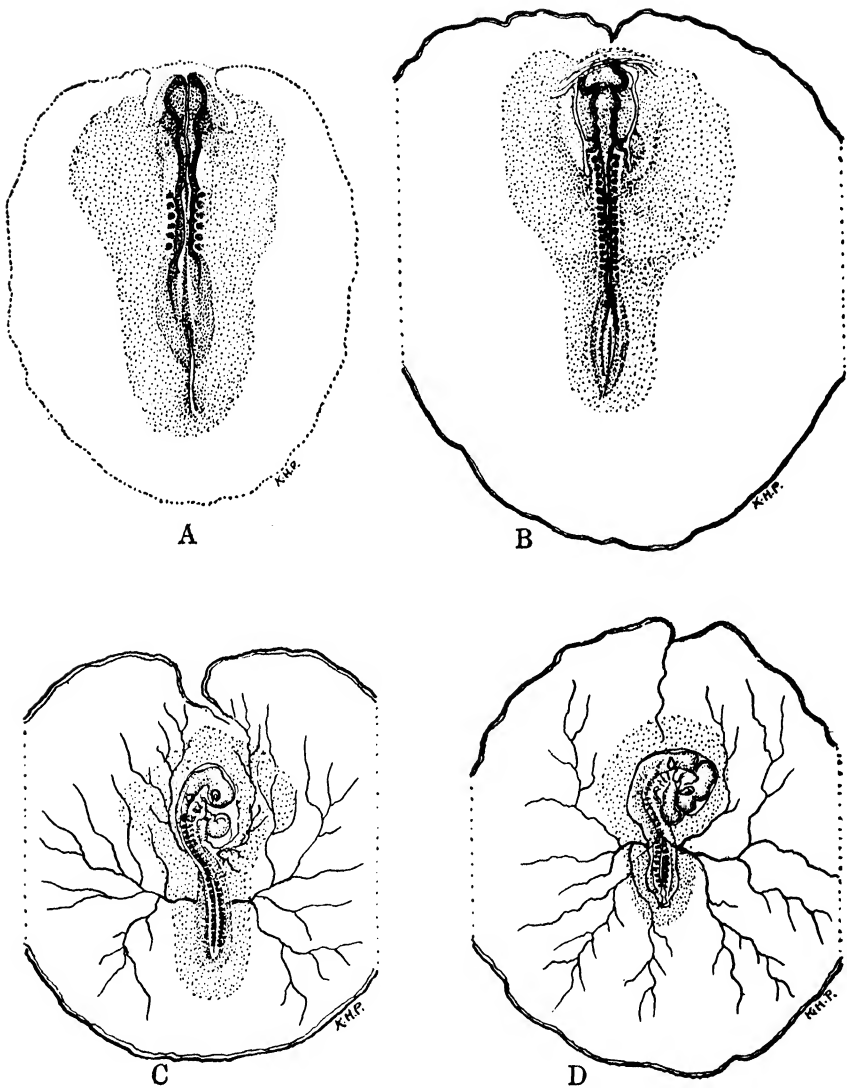


FIG. 85. — Growth of the chick embryo. A, 25 hours of incubation. B, 38 hours of incubation. C, 48 hours of incubation. D, 68 hours of incubation. Compare Figs. 200, 206, 212, 218, respectively. A, B, approx. $\times 9$; C, D, approx. $\times 4$. (After Duval.)

tion. Only the first three clefts actually open into the fore-gut, and these are soon closed again.

The tail arises from the backward growth of the tail fold but never attains any great length.

The limb buds appear during the third day of incubation.

BODY FORM IN MAN. — Human embryologists distinguish three periods during intra-uterine development: the period of the ovum, from fertilization to germ-layer formation, two weeks; the period of the embryo, until the embryo has assumed a definitely human appearance, the end of the second month; and the period of the fetus. It is the second of these with which we are concerned.

By the end of the third week the head fold is formed, and at the fifth the tail fold is developed. Neural folds are formed and unite

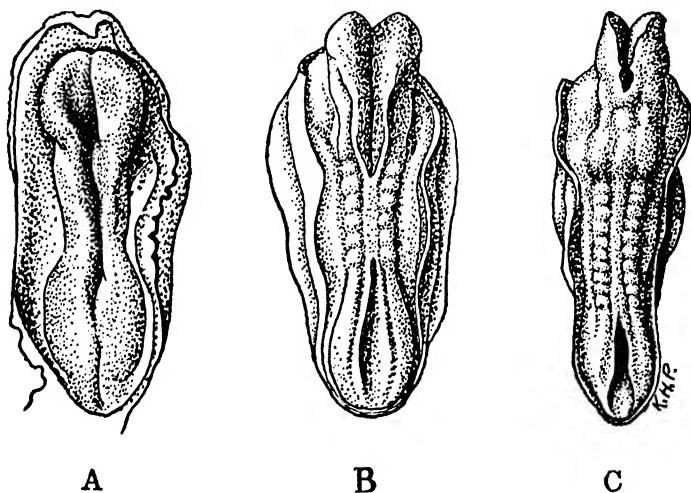


FIG. 86. — Growth of the human embryo. A, neural folds (after Ingalls). B, neural tube commencing, seven somites (after Payne). C, ten somites, (after Corner).

much as in the chick (Fig. 86). The primordia of eye, ear, and nose also originate in a similar manner. Five visceral grooves are formed, by the end of the fifth week, separating six visceral arches, but although the visceral pouches appear and unite with the grooves, true visceral clefts are not formed. By the end of the seventh week, the visceral grooves have disappeared. A cephalic flexure appears in the fifth week. The neck (cervical)

flexure develops in the week following and accelerates the disappearance of the visceral grooves.

A tail is developed from the tail fold which is quite prominent during the six and seventh weeks of development but is overgrown and resorbed during the eighth.

Limb buds make their appearance toward the end of the fifth week.

B. THE YOLK SAC

Yolk sacs are found in the development of all large-yolked eggs, among both anamniotes and amniotes. As the name implies, this structure is a larger or smaller bag protruding from the body and connected with it by a yolk stalk.

Origin and development. — The yolk sac develops from the outer margin of the blastoderm which advances under the vitelline membrane and around the yolk mass until the yolk is completely enclosed (Fig. 82).

Function and fate. — It contains the yolk, which, in meroblastic cleavage, is not divided among the blastomeres. But it plays a far more important part in development than simply acting as a reservoir for food reserves. (It is lined with endoderm just like that of the intestine, and is furnished with arteries, veins, and capillaries, which make up the area vasculosa. The endodermal lining digests the yolk, and the vitelline veins carry the digested food to the developing embryo. We may think of the yolk sac as an extra-embryonic intestine.) It is interesting to note that in some viviparous elasmobranchs, like the dogfish, the yolk sac continues to be of use, even after the yolk is consumed. Pressed against the wall of the uterus it absorbs the uterine "milk" which this organ secretes (much like a tertiary egg envelope) and conveys it to the embryo through the vitelline veins. A similar device is seen among the marsupials (page 144). The yolk sac is usually drawn up into the body when the umbilicus closes and is later resorbed.)

THE DISPOSITION OF THE YOLK IN THE FROG. — The frog has no yolk sac, for the yolk is divided among the large blastomeres which later make up the floor of the intestine. The mass of these cells, however, creates a bulge on the ventral surface of the embryo (Fig. 84) which resembles externally a small sac.

THE YOLK SAC OF THE CHICK. — The yolk sac of the chick is formed by the advancing edge of the blastoderm. Looking down on the blastoderm of the chick at the end of the first day of incubation (Fig. 79), one distinguishes a series of concentric rings. Proceeding from the periphery inward, we note first the area vitellina externa, consisting of the margin of overgrowth and the zone of junction (page 112). Then comes the area vitellina interna in which we can distinguish the ectoderm and endoderm, the latter closely applied to the yolk. Finally there is distinguished the area vasculosa into which the mesoderm has pushed, splitting, as it advances, into the somatic layer (next the ectoderm) and the splanchnic layer (next the endoderm). Between the somatic and splanchnic layers lies the exocoel (extra-embryonic coelom), as the coelom is called when it extends beyond the boundaries of the embryo. The blood vessels of the area vasculosa develop in the splanchnic mesoderm. The exocoel separates the splanchnopleure (endoderm and splanchnic mesoderm) from the somatopleure (somatic mesoderm and ectoderm), so that it can be said that the yolk sac of the chick consists of splanchnopleure. By the end of the fourth day of incubation the yolk is completely covered except for a small area at the vegetal pole, known as the yolk sac umbilicus (Fig. 89C, D). When the chick hatches, the empty yolk sac still attached to the intestine is drawn into the coelom and gradually disappears.

THE YOLK SAC OF MAN. — In man, as in other mammals, the yolk sac arises in connection with gastrulation. The endoderm growing out from the lower surface of the embryonic knob apparently reorganizes itself to form a very small gastrocoel or yolk sac. The roof of this gastrocoel forms the roof of the digestive canal; the anterior end is set off (with the head fold) to make the fore-gut; the posterior end is set off (with the tail fold) to make the hind-gut. The remainder constitutes the small yolk sac (Fig. 86A). This sac is later squeezed between the amnion and chorion (Fig. 90), and loses its connection with the intestine, through the degeneration of the yolk stalk.

In other mammals (Fig. 68) the endoderm grows completely around the interior of the trophoblast and forms a larger yolk sac. In the mouse, where the embryonic knob hangs well down in the cavity of the blastocyst, this results in the knob's being covered

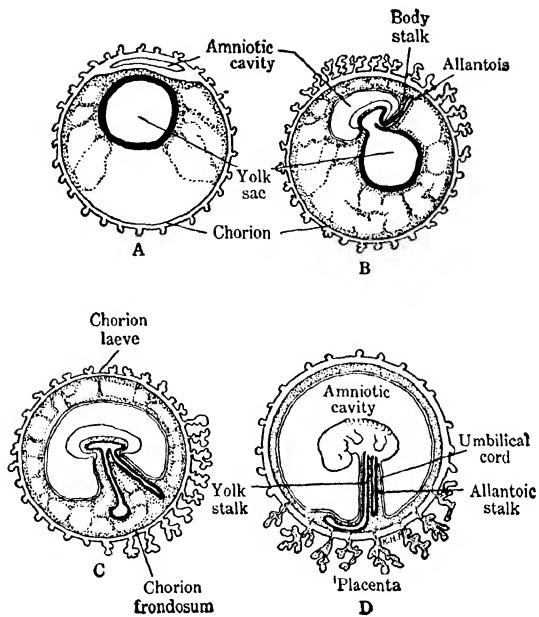


FIG. 90. — Diagrams to show development of extra-embryonic structures in human embryo. Four stages illustrated by sagittal sections. (After Corning.)

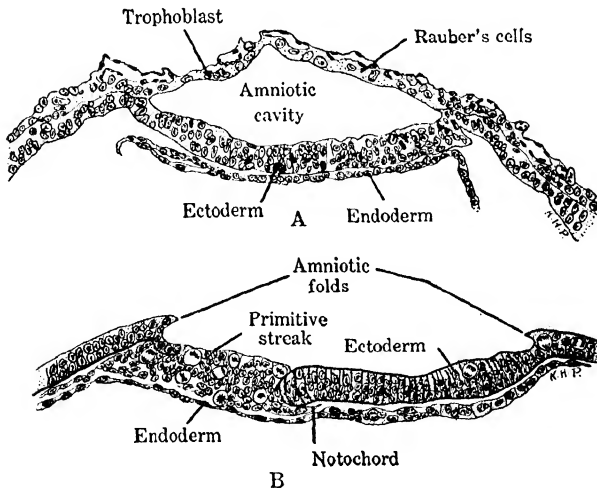


FIG. 91. — Amnion formation in the bat's egg. A, primary amniotic cavity. B, origin of amniotic folds. (After Van Beneden.)

ate. The embryonic disc thus comes to form part of the blastocyst wall.

The amnion and chorion are formed by amniotic folds (Fig. 91). The internal limb of each fold is formed of somatopleure derived from the embryonic disc and will form the amnion as in the chick. The outer limb of each fold, however, is formed of ectoderm derived from the trophoblast associated with somatic mesoderm and gives rise to the chorion. The mesoderm growing out from the primitive streak, and delaminating into somatic and splanchnic layers, becomes the lining of the exocoel.

D. THE ALLANTOIS

The development of an amnion and chorion is always accompanied by the appearance of another sac, the allantois. This extra-embryonic structure appears as an evagination from the hind-gut and is therefore lined with splanchnopleure. It grows out through the exocoel of the umbilical stalk into the exocoel of the chorion, which it usually fills. It is filled with an allantoic fluid which receives the nitrogenous wastes of the embryo in the form of uric acid (Needham), and may be thought of in the first instance as an extra-embryonic urinary bladder. As it fills the chorion, its walls, being composed of splanchnic mesoderm in the outer layer, easily fuse with the mesodermal layer of mesoderm of the amnion, chorion, and yolk sac, whenever these structures come together. Furthermore, it has an area vasculosa served by the allantoic (umbilical) veins and arteries. This area vasculosa when applied to the chorion is the region where the blood is nearest to a source of atmospheric oxygen. Here an exchange of gases, carbon dioxide for oxygen, takes place, and the allantois may be considered as an extra-embryonic lung.

In the cleidoic egg of reptiles, birds, and egg-laying mammals, the allantois also takes part in the formation of an albumen sac wherein this material is digested. In the marsupials and placental mammals it contributes to the formation of a placenta (hemiplacenta in marsupials) whereby digested food is obtained from the maternal circulation. These functions of the placenta will be discussed in the sections following.

ALLANTOIS OF THE CHICK. — The allantois (Fig. 92) arises towards the end of the third day as an evagination from the floor of

the hind-gut. It grows out between the yolk and the wall of the subcaudal pocket into the exocoel (Fig. 89B). Here it expands greatly until by the end of the ninth day it has filled the entire exocoel. Its outer wall unites with the chorion (Fig. 89C) to form a chorio-allantois, its inner wall unites with the amnion above and the yolk sac below.

Now the chorion, carrying with it an inner fold of allantois, grows down beyond the yolk-sac umbilicus (page 136), and around

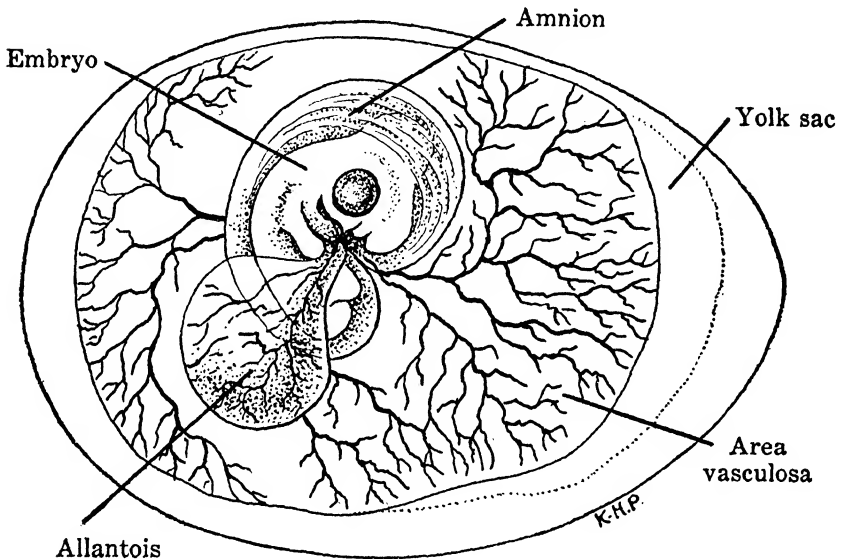


FIG. 92. — The embryo chick and its extra-embryonic structures on the sixth day of incubation. $\times 1\frac{1}{2}$. (After Duval.)

the mass of albumen, which has become more viscous through the loss of water and is displaced towards the lower side of the egg. The albumen is enclosed in a double-walled sac of chorion with the allantois between the two walls of the sac (Fig. 89D). The layer next to the albumen is the ectoderm of the chorion, but the mesoderm of the allantois supplies the blood vessels. It is interesting to observe that it is the ectoderm of the albumen sac which absorbs the albumen, whereas in the yolk sac it is the endoderm which carries on this function.

By the twelfth day of incubation the albumen sac is closed except at the yolk-sac umbilicus where it has an open connection

with the yolk sac. On the sixteenth day the albumen is consumed. On the seventeenth the yolk-sac umbilicus closes by the constriction of a ring of mesoderm derived from the old edge of the blastoderm. The yolk sac with the remains of the albumen sac still attached is retracted into the body cavity of the chick on the nineteenth day of incubation, aided by contractions of the amnion and the inner wall of the allantois.

ALLANTOIS OF MAN AND OTHER MAMMALS. — In most of the mammals there is a well-developed allantois, arising like that of the chick, growing into the exocoel, and uniting with the chorion to participate in the formation of the placenta, but the human allantois is rudimentary. It arises as a minute tubular evagination which develops from the endodermal roof of the gastrocoel even before the formation of the tail fold. It grows out into the body stalk, a mass of mesoderm connecting the embryo with the chorion (Fig. 90) for a short distance, but never gets so far as the chorion. However, the allantoic (umbilical) blood vessels continue down the body stalk to the chorion where they form a chorio-*nic area vasculosa* in the region of the developing placenta.

THE PLACENTA

Before discussing the human placenta it will be helpful to review the different types of placentation recognized in mammals. Two types are distinguished according to the degree of union between the trophoblast and the lining of the uterus (*mucosa*); a second basis of distinction is whether the wall of the allantois comes in contact with the chorion or not.

Indeciduate type. — The first type of placenta is called indeciduate. In this type, found in several groups particularly the ungulates, the trophoblast is closely applied to the *mucosa* but both retain their integrity. The blood vessels of the placenta absorb food material excreted by the *mucosa* and exchange carbon dioxide for oxygen by diffusion.

Marsupials. — Among the marsupials are found both non-allantoic and allantoic hemiplacentae. In the opossum, *Didelphys* (Fig. 93A), the enlarged yolk sac is pressed against the trophoblast, which in turn is closely applied to the *mucosa*, forming folds which project into depressions in the uterine wall. The absorbed nutriment is conveyed to the embryo by means of

the area vasculosa of the yolk sac. In *Perameles* (Fig. 93B), an allantoic hemiplacenta is formed by the union of the allantoic sac with the trophoblast. Where this hemiplacenta touches the

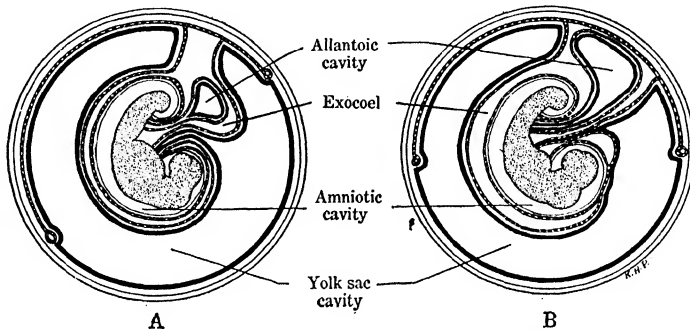


FIG. 93. — The extra-embryonic structures of marsupials. Diagrammatic. A, *Didelphys*. B, *Perameles*. (After Jenkinson.)

mucosa the epithelium of the latter thickens and is invaded by maternal capillaries. The trophoblast is said to be resorbed so that the capillaries of the allantois come into intimate connection with those of the uterus. It should be mentioned in this connection that *Perameles* also possesses a well-developed area vasculosa

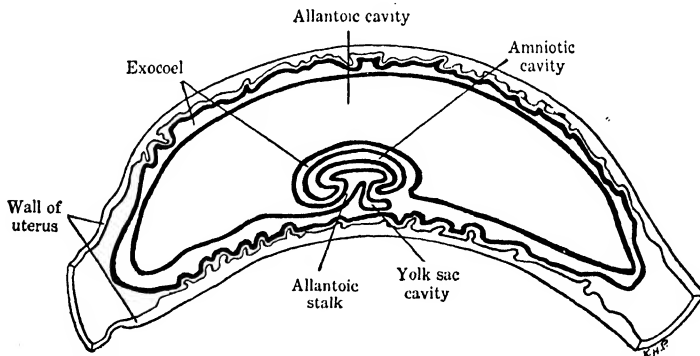


FIG. 94. — Diagram of extra-embryonic structures in the pig. (After Smith.)

on the yolk sac. It is very probable, therefore, that both yolk sac and allantoic circulations are concerned with the nutrition of the developing young.

Ungulates. — In the ungulates there is a well-developed allantoic placenta of the indeciduate type (Fig. 94). The blastocyst elongates, and over its surface appear projections of the tropho-

blast which contain a core of mesoderm. These projections, villi, grow into corresponding depressions of the uterine wall, called crypts. The allantois meantime has filled the exocoel, and capillaries from the allantoic arteries and veins penetrate the mesodermal cores of the villi. These capillaries are brought very near those of the uterine wall, but the blood remains separated from that of the mother by (1) the endothelial lining of the maternal capillaries, (2) the connective tissue of the mucosa, (3) the epithelium of the mucosa, (4) the trophoblast, (5) the mesoderm of the villi, and (6) the endothelial lining of the fetal capillaries (Fig. 99A, B). At birth the villi are pulled out of the crypts, and the placenta, with the remaining embryonic membranes, is discharged as the "after-birth."

Deciduate type. — The second type of placentation is called deciduate. In this type the trophoblast attacks the mucosa and

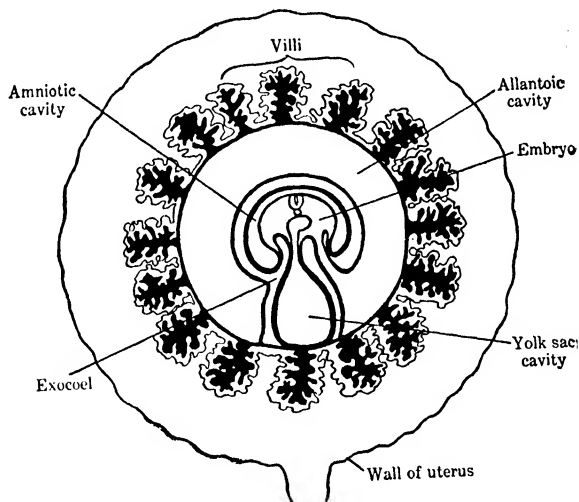


FIG. 95. — Diagram of extra-embryonic structures in the dog. Sagittal section. (After Jenkinson.)

erodes part of the lining. It is characteristic of the majority of the clawed mammals (unguiculates) and primates. In the first group the placenta is allantoic; in the primates, non-allantoic.

Carnivores. — In the carnivores (Fig. 95) is found a deciduate placenta of the allantoic type. The blastocyst elongates although not to the extent it does in the ungulates. During this time

the epithelium of the uterus is cast off. At the circular zone of the uterus which is in contact with the equator of the blastocyst the epithelium of the uterus fails to regenerate. Into this zonary area grow the villi of the trophoblast which become penetrated by the allantoic capillaries. The villi send out branched processes, each with its capillaries, which surround the maternal capillaries. Thus the maternal blood is separated from that of the fetus by (1) the endothelium of the maternal capillaries, (2) a varying amount of maternal connective tissue, (3) the trophoblast, (4) a varying amount of chorionic connective tissue, and (5) the endothelial lining of the fetal capillaries (Fig. 99C). At birth a certain amount of maternal tissue is torn away with the placenta.

PLACENTA OF MAN. — In the human placenta there is the most intimate contact between the maternal and fetal circulation.

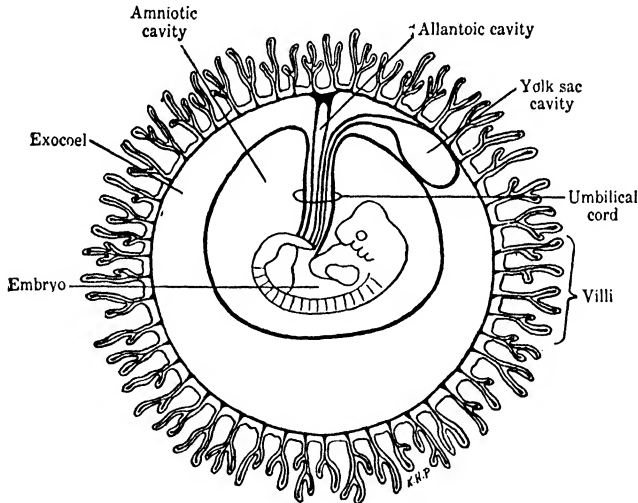


FIG. 96. — Diagram of extra-embryonic structures in man. (After Kölliker.)

The placenta is non-allantoic. It will be recalled that the embryonic knob retains its connection with the trophoblast as the body stalk. Into the body stalk grows the small evagination from the hind-gut which represents the endodermal lining of the allantois (Fig. 90). It never comes in contact with the trophoblast and soon degenerates. The limiting sulci of the amnion approach each other and become the walls of the umbilical cord.

This encloses (Fig. 96) the body stalk, yolk stalk, allantoic stalk, as well as the two umbilical arteries and two umbilical veins which

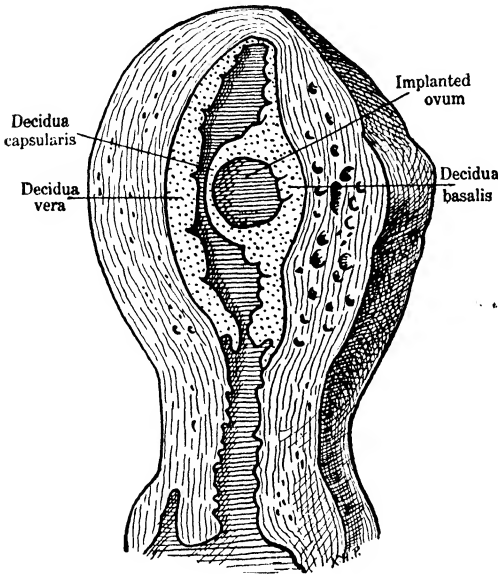


FIG. 97. — Diagram to show the uterine deciduae (human). (After Kollmann.)

grow out from the body of the embryo towards the trophoblast. These umbilical blood vessels represent the allantoic vessels of all other amniotes. Later the umbilical veins fuse, and all this tissue assumes common connective tissue characteristics with the exception of the walls of the blood vessels.

The deciduae. — It will be remembered that the blastocyst burrows into the uterine wall, eroding epithelium, connective tissue, and blood vessels. As the embryo increases in size, this erosion continues and the embryo sinks into the compact layer of the mucosa and comes in contact with the spongy layer. The mucosa grows around the burrowing embryo, shutting it off from the cavity of the uterus. There may now be distinguished (Fig. 97) three regions in the mucosa: (1) the decidua basalis, to which the blastocyst is attached; (2) the decidua capsularis, which cuts off the blastocyst from the uterine cavity; and (3) the decidua vera, including the remainder of the uterine lining.

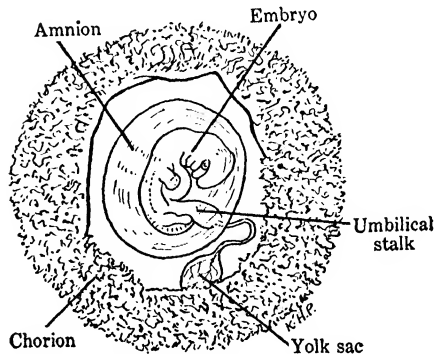


FIG. 98. — Human embryo 11 mm. in length, about 6 weeks old, to show extra-embryonic structures. $\times 1\frac{1}{2}$. (After Arey.)

The chorion. — The trophoblast, while entering the uterine wall, becomes differentiated into an outer syncytial layer and an inner cellular layer. During the process of implantation, nutrition is obtained by the syncytial layer, which sends out projections or false villi into the maternal tissue. Thereafter mesodermal cores grow into the false villi converting them into the true villi which later receive capillaries from the umbilical blood vessels.

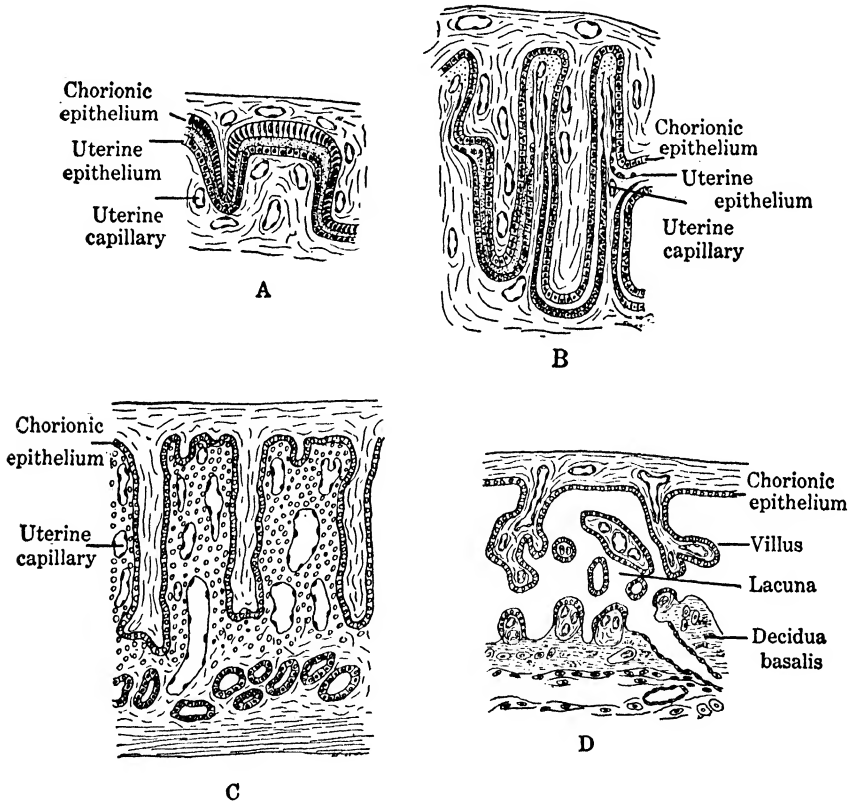


FIG. 99. — Sections through placentae of A, pig; B, cow; C, cat; and D, human. (Semi-diagrammatic after Grosser.)

Some of these bore into the uterine wall to become fixation villi. The others, losing their syncytial layer, remain in the space between the trophoblast of the chorion and the maternal tissue as nutrition villi (Fig. 98). These are bathed in maternal blood which is brought into the intervillous space and carried thence by the eroded uterine capillaries. Only those villi which are in

contact with the decidua basalis persist; the others degenerate, thus differentiating the chorion into the chorion frondosum, with villi, and the chorion laeve, devoid of the same. In the human

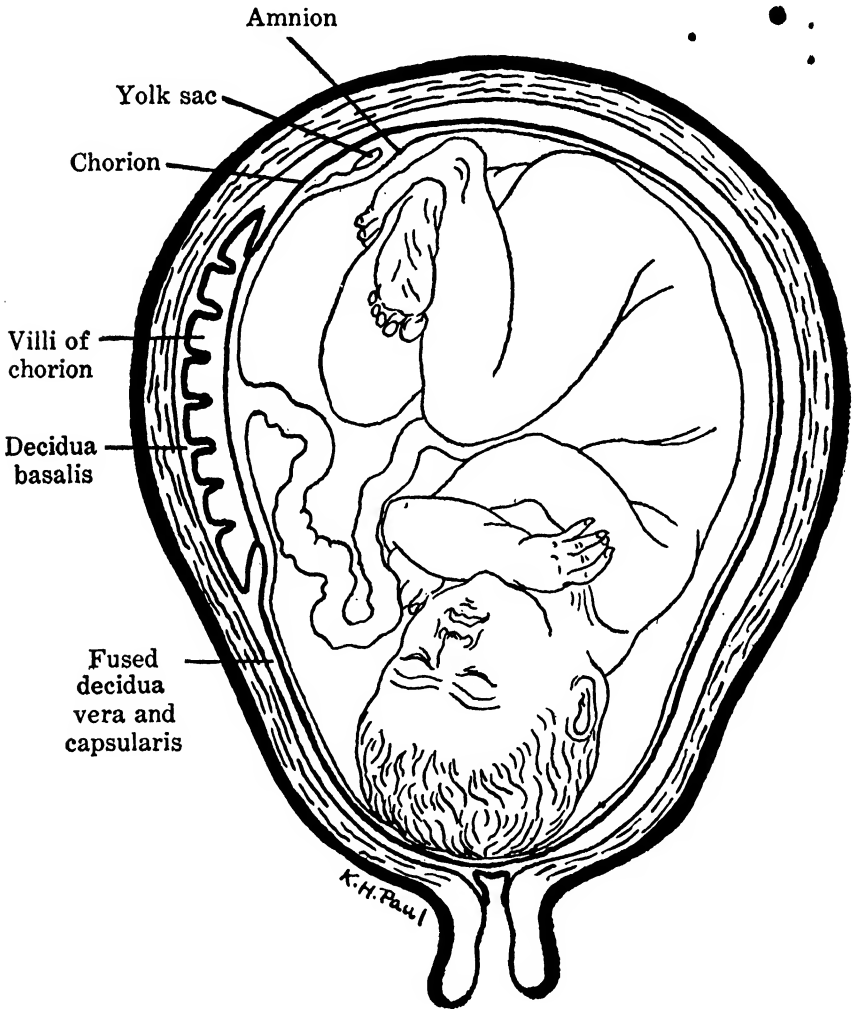


FIG. 100. — Diagram of fetus (near term) to show relationships of extra-embryonic structures and deciduae. (After Ahlfeld.)

placenta the maternal blood is separated from the fetal blood stream by only (1) the cellular layer of the trophoblast, (2) the chorionic connective tissue of the villi, and (3) the endothelia of the fetal capillaries (Fig. 99D).

Parturition. — The history of the extra-embryonic structures as well as that of the deciduae is terminated by birth (parturition). Owing to the absence of an allantoic sac the amnion enlarges to fill the exocoel. Later, growth of the fetus results in pressing the chorion laeve and decidua capsularis against the decidua vera and obliterating the uterine cavity (Fig. 100). At birth the placenta, carrying with it the decidua basalis, and the attached membrane, which represents the fused amnion, chorion laeve, decidua capsularis, and decidua vera, are cast off as the caul or "after-birth."

SUMMARY

The method by which the external form of the vertebrate embryo is assumed is closely connected on the one hand with the shape of the gastrula, and on the other with the presence or absence of certain extra-embryonic structures, the yolk sac, amnion, chorion, and allantois.

With growth in length we associate the occurrence of metamerism, or the serial repetition of parts, and the formation of a head and a tail. The paired limbs arise as buds.

The yolk sac is found only in embryos developing from extremely telolecithal eggs. It is lined with endoderm and functions as an extra-embryonic intestine. The splanchnic layer of the mesoderm adjacent to it develops an area vasculosa which conveys the digested yolk to the body of the embryo.

The amnion and the chorion arise typically from folds of somatopleure which fuse above the embryo, thus giving rise to an inner membrane, the amnion, and an outer one, the chorion. The amnion, lined with ectoderm internally, contains amniotic fluid in which the embryo develops. The chorion, lined with somatic mesoderm internally, contains the exocoel, a continuation of the embryonic coelom. Neither of these membranes has any vascular system of its own. They are found only in the development of reptiles, birds, and mammals.

The allantois always develops in amniote embryos. It arises as a ventral evagination of the hind-gut and typically grows out into the exocoel which it completely fills. It functions as an extra-embryonic bladder and lung, and because of its vascular area may act (in connection with the chorion) as an organ of nutrition, e.g., as an albumen sac.

In mammals the blood vessels of the allantois invade the chorion giving rise to the placenta, an organ where substances may be exchanged by diffusion between the maternal and fetal blood streams. The placenta is connected to the embryo by the umbilical stalk, whose walls are formed by the amnion. In some mammals, such as the carnivores and primates, parts of the uterine wall, the deciduae, are concerned in the formation of the placenta, and cast off with them at birth.

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CHAPTER VII

EXPERIMENTAL VERTEBRATE EMBRYOLOGY

Recent progress in vertebrate embryology has resulted so largely from the application of the experimental method that even the beginning student must acquaint himself with some of the methods used and the results so far obtained. Within the limits of this text only a few of the important fields in which the experimental method has been employed can be mentioned, and the student must be referred to more extended treatises for further information concerning this relatively new and important branch of embryology.

The amphioxus and the frog have long been used by experimental embryologists, and more recently successful methods have been devised for the experimental study of the developing egg of the hen. *Triton*, in Germany, and *Ambystoma*, in this country, are urodele amphibia whose eggs have been particularly favorable for experimental embryology. The eggs of mammals, difficult to obtain, and, so far, impossible to orient during the early stages of embryology, have been employed to a lesser extent.

The experimental embryologist alters the conditions under which the egg develops in the hope of determining the factors involved in particular developmental processes. It is appropriate that we conclude our study of early embryology with a short account of some of the experiments which bear directly on the organization of the fertilized egg, on differentiation during cleavage and the formation of the germ layers, and on the direct effects of environmental factors upon development.

A. THE ORGANIZATION OF THE FERTILIZED EGG

The fertilized egg, as we have seen, is the product resulting from the union of two germ cells, the egg and the sperm. It contains two pronuclei, of maternal and paternal origin, respectively, as well as a mass of cytoplasm which is almost exclusively maternal in origin. The nuclei contain the parental

contributions of genes, the units which together determine the hereditary characters of the developing individual. How the genes produce their effects is not known, but it is certain that they must act directly upon the cytoplasm. Accordingly we may turn first to experiments dealing with the nuclei of the fertilized egg, and second, to those concerned with the organization of the cytosome itself.

1. THE NUCLEAR ORGANIZATION

The fact that the fertilized egg has the diploid number of chromosomes and of genes, while the two gametes have the haploid number, naturally leads to the question whether the diploid number is necessary to continued development. A considerable number of experiments bear directly upon this question.

Artificial parthenogenesis. — The frog's egg can be induced to develop by puncture with a finely pointed glass needle (Loeb and others). These artificially parthenogenetic eggs have given rise to tadpoles and frogs. Apparently the number of chromosomes is redoubled (diploid number), perhaps by a division of the chromosomes without a corresponding division of the cell. But the genes are exclusively maternal in origin.

Irradiated sperm. — Sperms of the amphibian *Triton*, treated to an appropriate dosage of radium emanations, have their nuclei injured in such a way that they are unable to form normal pronuclei (Hertwig). But they retain their mobility and are able to penetrate the egg and induce development. The sperm head remains in the cytoplasm and passes to one or another of the developing blastomeres but takes no part in mitosis and ultimately degenerates. The number of chromosomes in the larval cells is usually haploid, although redoubling may occur.

Irradiated eggs. — Eggs of *Triton* have also been irradiated to kill the egg nucleus and then fertilized with normal sperms. These eggs develop with the haploid number of chromosomes, showing that either pronucleus, maternal or paternal, is adequate for development.

Fertilization of enucleate eggs. — In some marine invertebrates, e.g., the sea urchin, the egg can be broken into fragments by shaking. Naturally only one fragment will contain the nucleus, but the enucleate fragments can be fertilized and will

give rise to dwarf but otherwise normal larvae. This phenomenon is known as merogony. A similar result can be obtained in telolecithal vertebrate eggs such as those of *Triton*, where several sperms normally enter the egg. After the entrance of the sperm

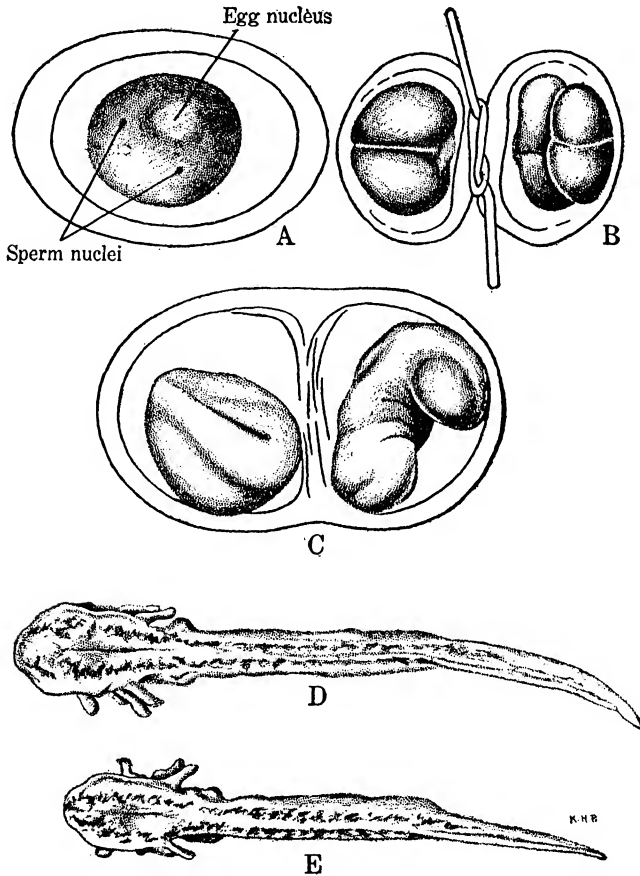


FIG. 101. — The experimental production of haploid larvae in *Triton*. A, fertilized egg with two sperm nuclei. B, same after constriction separating part of egg with diploid nucleus (right) from part with haploid nucleus formed by supernumerary sperm (left). C, showing relatively more advanced diploid embryo (right) and less advanced haploid embryo (left). D, diploid larva. E, haploid larva. (After Spemann.)

it is possible to constrict the egg into two halves, by means of a fine hair loop, in such a way that the female pronucleus lies in one half (Fig. 101). This half will eventually have the diploid number of chromosomes, for a sperm pronucleus will conjugate with

the egg pronucleus. The other half will have only the haploid number. Both halves will develop into larvae, one of which will have haploid and the other diploid nuclei.

Species hybrids. — Many experiments have been made in the attempt to fertilize the egg of one species with a sperm from another species. Often as in the teleost fish (Moenkhaus), both pronuclei take part in the subsequent cleavage, although frequently the chromosomes from the two pronuclei (Fig. 102) form separate groups on the mitotic spindle (gonomery). But in other cases Hertwig has shown that the male pronucleus takes no part in subsequent cleavages, so that the embryo really develops parthenogenetically.

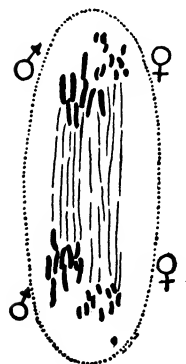


FIG. 102. — Chromosomes in anaphase of first cleavage of a hybrid fish, *Menidia* egg and *Fundulus* sperm, illustrating gonomery. (After Moenkhaus.)

Natural interspecific hybrids in both plant and animal kingdom are more common than formerly believed. Usually these interspecific hybrids are infertile, as the mule and many types of hybrid bony fish, but they often grow to larger size and are more active (hybrid vigor) than the parents.

The equivalence of the pronuclei. — Although, as we have seen in Chapter IV, the pronuclei may differ from one another in regard to individual genes, the experiments mentioned above indicate that a single set of genes, paternal or maternal, is adequate for the development of an egg. It must be recognized that the experimental haploid animals are frequently less vigorous than normal diploid forms.

2. THE ORGANIZATION OF THE CYTOPLASM

Polarity. — The primary expression of the egg's organization is the polarity already impressed upon it in the ovary (page 37). That this polarity is itself not due to gravity is shown by the fact that frog eggs which are kept in motion during early development give rise to normal embryos (Morgan, Kathariner). But polarity is not immutable, for many experiments in which the eggs of frogs have been made to develop in an inverted position (Born, Pflüger, Morgan) show that the yolk streams down through the egg, and

cleavage begins in the relatively yolk-free region which was formerly the vegetal pole.

Gradient. — There seems to be good reason to suppose that the polar axis represents a metabolic axial gradient (Child), for when dilute solutions of lethal chemicals, e.g., potassium cyanide, are applied to the frog's egg (Bellamy), disintegration begins at the animal pole and continues toward the vegetal pole, which is the last part of the egg to be affected.

Cytoplasmic materials. — In some animals there seems to be a definite stratification of materials in the egg along the polar axis, but when this stratification is disturbed by whirling the eggs about in a centrifuge, the eggs develop with the original polarity undisturbed. On the other hand, in telolecithal eggs like that of the frog, centrifuging distorts the cytoplasmic framework (Conklin).

Bilaterality. — The animal pole marks the anterior end of the developing amphibian embryo. Its dorsal side is marked by the gray crescent which appears on the side opposite the point of entry of the sperm. Many observations (Jenkinson and others) show that the point of entry marks a second dorso-ventral axis and establishes the bilaterality of the developing embryo. But in parthenogenetic eggs (when development is initiated by puncture) the point of entrance of the needle seems to have no constant relation to the subsequent bilaterality of the egg. This would indicate (Huxley and de Beer) that the egg has an underlying bilaterality of its own which is not strong enough to withstand the stronger stimulus afforded by the entrance of the sperm but is apparent in parthenogenesis.

Bellamy has described a second axial gradient in the frog's egg shown by the action of potassium cyanide in which the high point centers in the gray crescent. This is the dorso-ventral axis of the embryo, which is therefore normally determined by the entrance point of the sperm.

Asymmetry. — The vertebrate embryo is not, strictly speaking, bilaterally symmetrical. A third axis or gradient from one side to the other (usually left to right) is often apparent, as seen in the development of the atriopore on the left side of the tadpole, the fact that the heart of the chick develops on the right side, and the fact that the head turns to the right in torsion. The stomach in

all vertebrates is twisted to the left of the mid-line, and many other examples might be mentioned. When this asymmetry is reversed we have the phenomenon known as situs inversus, and this condition can be reproduced experimentally by developing the egg in a lateral temperature gradient and in other ways. Thus the egg of the hen when overheated on the left side develops situs inversus. It has been shown by Spemann that, when two blastomeres which would ordinarily produce the right and left sides of an embryo are separated by a hair loop, the left-hand blastomere gives rise to a normally asymmetrical embryo, while the right-hand blastomere gives rise either to an embryo with normal asymmetry or to one with situs inversus.

These few examples of experiments on the fertilized egg indicate that the egg is a complex system with a definite organization indicated by its three axial gradients corresponding to its three spatial dimensions, viz., an antero-posterior gradient (polarity), a dorso-ventral gradient, and frequently a left-right gradient. Furthermore, the system contains two complete sets of chromosomes and genes, either one of which is adequate in further development.

B. ORGANIZATION OF THE EMBRYO DURING CLEAVAGE

Cell-lineage studies seemed to indicate that the dividing egg is becoming a mosaic of blastomeres, each set apart from the others to form a specific portion of the embryo. Roux (1888) was the first to realize that this might be tested experimentally. He destroyed one of the $\frac{1}{2}$ -blastomeres of the frog's egg and observed that the other gave rise to a $\frac{1}{2}$ -embryo, which later regenerated the missing portion.

Later investigators devised a number of methods by which blastomeres could be separated from each other, by shaking them, cutting them apart with fine needles, constricting them with fine threads, or placing them in artificial calcium-free sea water. Blastomeres of marine eggs in this medium separate immediately, and when returned to normal sea water continue their development without further separation (Herbst).

Regulation and mosaic eggs.—The results of their experimentation seemed to indicate that in some eggs, e.g., those of the amphioxus (Fig. 103), either of the $\frac{1}{2}$ -blastomeres might, when

separated, give rise to complete embryos (Wilson). These were called regulation eggs and were said to have indeterminate cleavage. In others, such as *Styela* (Conklin) or the mollusc *Dentalium* (Wilson), the $\frac{1}{2}$ -blastomeres give rise only to $\frac{1}{2}$ -embryos (Fig. 103). These were called mosaic eggs and were said to have determinate cleavage.

Experiments on frog's eggs had been inconclusive until recently an improved technique has made it possible to separate blastomeres of the two-cell stage completely (Schmidt, 1930, 1933).

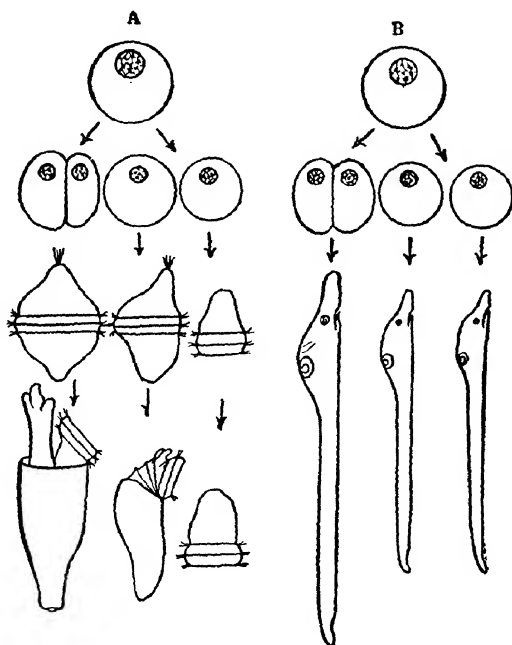


FIG. 103. — Diagram to show the fate of isolated blastomeres from mosaic and regulation eggs. A, mosaic egg of *Dentalium*. At left, a complete embryo produced by entire egg; at right, partial embryos produced by the $\frac{1}{2}$ -blastomeres when artificially separated. B, regulation egg of *Amphioxus*. At left, embryo produced by entire egg; at right, perfect dwarf embryos produced by $\frac{1}{2}$ -blastomeres. (After Wilson.)

These experiments show that each of the $\frac{1}{2}$ -blastomeres can give rise to a complete and perfect larva, provided only it contains some of the gray crescent region. If, on the other hand, the egg is so constricted that the first cleavage divides it into an animal and a vegetal half, the animal half, containing the gray crescent,

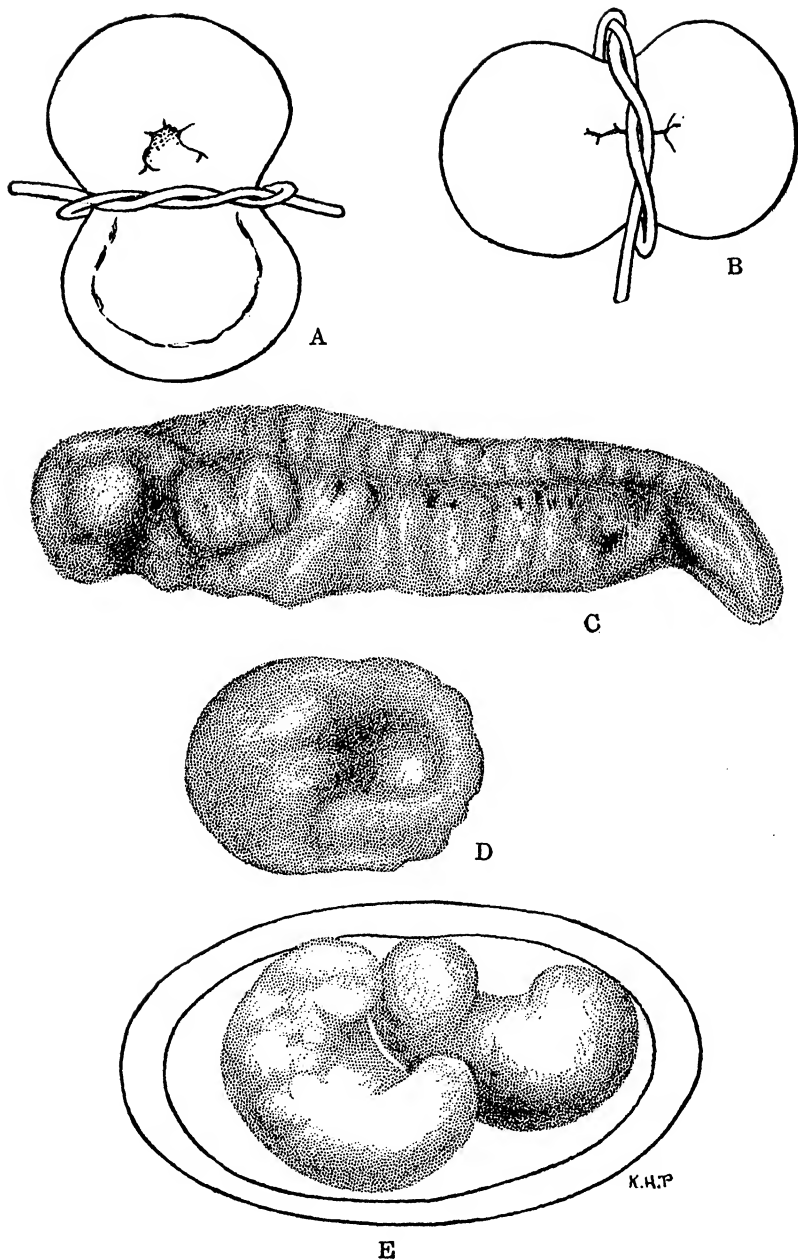


FIG. 104. — Embryos arising from separated $\frac{1}{2}$ -blastomeres of the newt's egg. A, the constriction separates the dorsal and ventral halves of the embryo. B, the constriction separates the right and left halves. C, perfect embryo arising from the dorsal $\frac{1}{2}$ -blastomere. D, mass of cells arising from ventral $\frac{1}{2}$ -blastomere. E, two perfect embryos arising from right and left $\frac{1}{2}$ -blastomeres respectively. (After Spemann.)

gives rise to a complete embryo, while the vegetal half, lacking this region, is unable so to organize itself (Fig. 104). The importance of the gray crescent as the seat of the organizer is discussed on page 169. This seems to indicate that Roux's results were due to the presence of the injured blastomere inhibiting complete development on the part of the uninjured blastomere. In this connection it is interesting to note that Witschi (1927) has

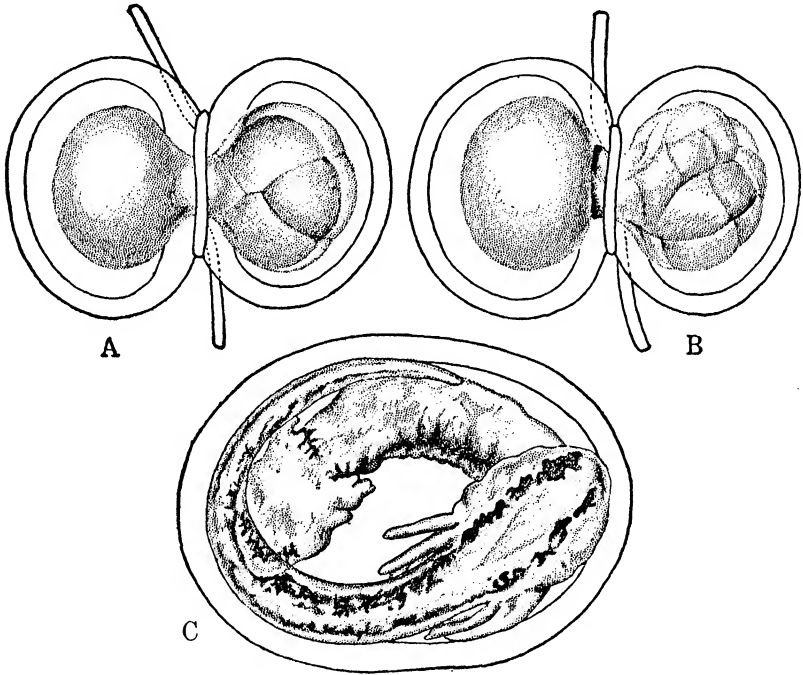


FIG. 105. — Experiment demonstrating equality of nuclei formed during cleavage (*Triton*). A ligature has been tied around the fertilized egg restricting the nucleus to the right-hand portion. A, 16-cell stage, one nucleus passing into left-hand portion. B, ligature tightened to separate the two portions. C, perfect embryos formed by the separate portions. The nucleus of a $\frac{1}{16}$ th-blastomere equivalent to that of a complete zygote. (After Spemann.)

described a case in which two eggs were found in a single chorion. Each of them was flattened on the side next to its neighbor and in later development showed deficiencies in the corresponding region.

A beautiful demonstration that it is the cytoplasm and not the nucleus which is concerned with differentiation during cleavage is afforded by an instructive experiment of Spemann. If the egg

is tied off before cleavage so that the nucleus is confined in one of its halves (Fig. 105), all cleavage planes will be restricted to that half until eventually a cleavage plane, in this case at the fourth cleavage, coincides with the plane of constriction. The nucleus which enters the previously enucleate half is naturally one which would serve a $\frac{1}{16}$ -blastomere. If the loop is now tightened until the two halves are completely separated, the portion containing this single nucleus will give rise to an embryo like the one from the portion containing the fifteen nuclei and exactly like one arising from a complete fertilized egg.

Pressure experiments. — Further examples of the regulative power of some eggs may be seen in pressure experiments. If the eggs of the frog are placed between glass plates during cleavage, the third cleavage planes will be meridional instead of latitudinal, and the fourth cleavage plane is latitudinal (Fig. 106). Now if

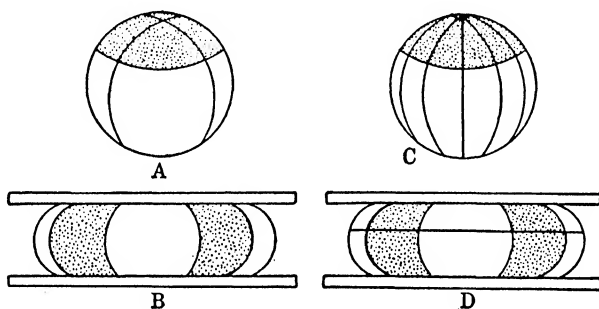


FIG. 106. — Diagram to show new relationship of blastomeres in frog's egg resulting from pressure during cleavage. A, normal 8-cell stage. B, 8-cell stage formed under pressure. C, normal 16-cell stage. D, 16-cell stage formed under pressure. Cells normally in animal hemisphere shown in stipple. (Suggested by a diagram in Wells, Huxley and Wells.)

the eggs are released, their later development will be quite normal even though the blastomeres are occupying positions unlike those which they hold ordinarily.

Double embryos. — Still another example may be seen in the eggs of *Triton*. If these are freed from the egg envelopes, the blastomeres at the two-cell stage assume a dumb-bell shape. Mangold discovered that, by placing one embryo in the two-cell stage over another (Fig. 107), a double embryo resulted almost exactly similar to a single embryo in the four-cell stage, and would

develop as such, provided only that the gray-crescent regions of the two fell in the same plane. Otherwise double monsters resulted. We shall see the importance of the gray-crescent region more clearly in a later section dealing with the organizer which develops in this region.

Chemo-differentiation. — It is quite clear from these experiments that the developing egg of the regulation type possesses a very great plasticity in the early stages of development as compared to the mosaic type illustrated by the egg of the tunicates. It may be assumed that the difference between these two types lies in the time at which definite organ-forming substances are segregated in the cytoplasm of the egg. Conklin has demonstrated that these regions are segregated after fertilization in the egg of the tunicate, whereas in amphibian eggs the only segregated region is that of the gray crescent. Huxley (1924) has suggested the term chemo-differentiation for the segregation of organ-forming substances. A good example is seen in the first division of the egg of *Dentalium*, the mollusc referred to above where a polar lobe passes completely to one or the other of the first $\frac{1}{2}$ -blastomeres. The cell receiving this lobe gives rise to the apical organ, mesoderm, foot, and shell. Here the very first division of the fertilized egg is determinate and dependent upon the segregation of the organ-forming substance found in the polar lobe (Fig. 108).

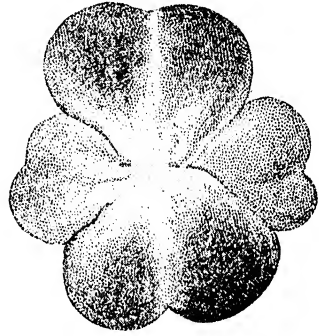


FIG. 107. — Double embryo arising from fusion of 2-cell stages of *Triton alpestris* (above and below) and *Triton taeniatus* (right and left) when laid over each other crosswise. Note that a new cleavage is under way in all blastomeres. (After Mangold and Seidel.)

Monovular twins and monsters. — The extreme plasticity of the vertebrate egg as seen by the fact that either two separate individuals or duplicate monsters may be formed from the complete or partial separation of blastomeres suggests an explanation of identical twins and the duplicate monsters which play so large a part in the study of teratology. It is generally accepted that identical, as distinguished from fraternal, twins are the product

of a single fertilized egg which has divided completely during early embryology, whereas the duplicate monsters, ranging from Siamese twins to monsters in which one individual is but a parasite upon the body of the other, result from incomplete separation. These identical twins are always of the same sex. Ordinary or

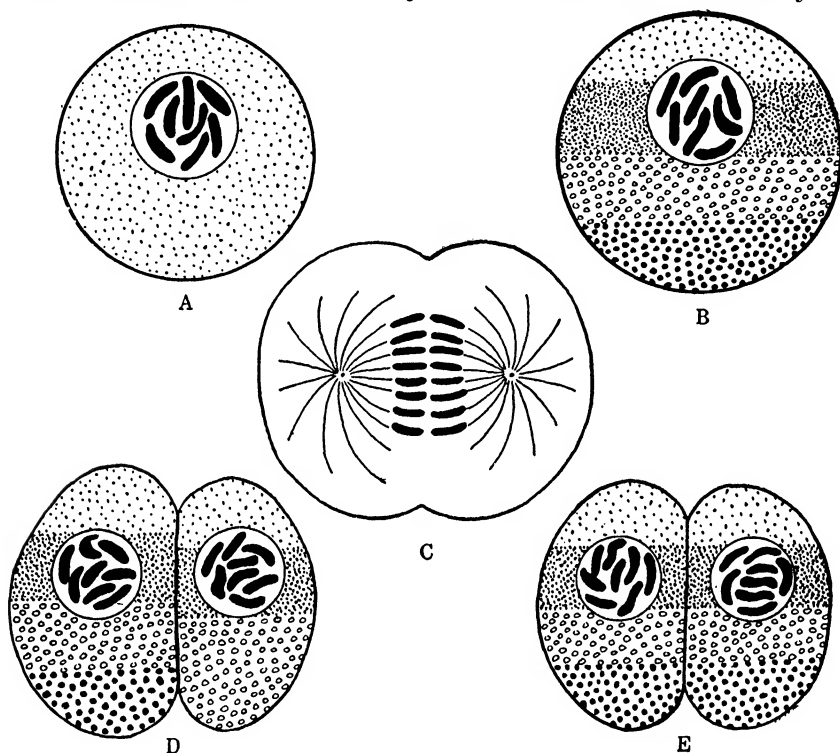


FIG. 108. — Diagram to show possible distribution of organ-forming substances in mosaic and regulation eggs. A, immature egg. B, mature egg showing stratified organ-forming substances. C, cleavage with equal division of chromosomes. D, segregation of one organ-forming substance in left-hand $\frac{1}{2}$ -blastomere. E, equal division of organ-forming substances between the $\frac{1}{2}$ -blastomeres. (After Wilson.)

fraternal twins (triplets, etc.) are supposed to be the product of separate eggs which ovulated and were fertilized at about the same time. Such twins are frequently of different sexes. In this connection we might mention the free-martin, a sterile female twinned with a male, not infrequent among cattle, and supposed to result from one of two eggs which develop a common chorion and therefore a common blood stream. It is supposed that a male

hormone circulating in the common blood stream inhibits the normal development of the female twin, so resulting in the production of the sterile free-martin (Lillie).

C. ORGANIZATION OF THE EMBRYO DURING GERM-LAYER FORMATION

The amphibian embryo is remarkably hardy and during the early stages of development will endure very severe operations. The work of Harrison in this country and of Spemann in Germany has resulted in the perfection of a method of removing portions of an embryo (micro-dissection) and grafting them into a new environment, where they will continue development. The embryo from which the portion is removed is known as the donor; the removed portion is called the graft (transplant); and, when the portion removed is transplanted into another embryo, the second embryo is termed the host.

The accompanying diagram (Fig. 109) will bring out some of the methods which have been developed in transplantation experiments. Thus the graft may be transplanted into another portion of the same embryo (homoplastic transplantation).¹ It may be transplanted into another embryo of the same species (heteroplastic transplantation). It may even be transplanted into an embryo of another species or genus (xenoplastic transplantation).

Another method which has brought interesting results is to transplant the removed portion into a nutrient medium and allow it to develop there under sterile conditions (explantation). This is also known as cultivation "in vitro," which means in glass. Another ingenious technique is to transplant the graft into a cavity of another embryo and allow it to develop there. The example shown in the diagram is of a bit of embryonic tissue transplanted into the eyeball of a tadpole, which acts as a nutrient chamber. Hoadley and others have developed a technique of grafting chick-embryo tissue from a donor to the chorio-allantois of a host. Such a technique is called interplantation (implantation).

Plasticity (dependent differentiation). — In the amphibian egg, which is of the regulation type, it has been demonstrated that the

¹ Some investigators use autoplastic=homoplastic; homoplastic=heteroplastic; and heteroplastic=xenoplastic.

presumptive organ regions of the blastula, (and until about the middle of gastrulation) are quite plastic, i.e., can be transplanted into other localities and will give rise to the organs appropriate

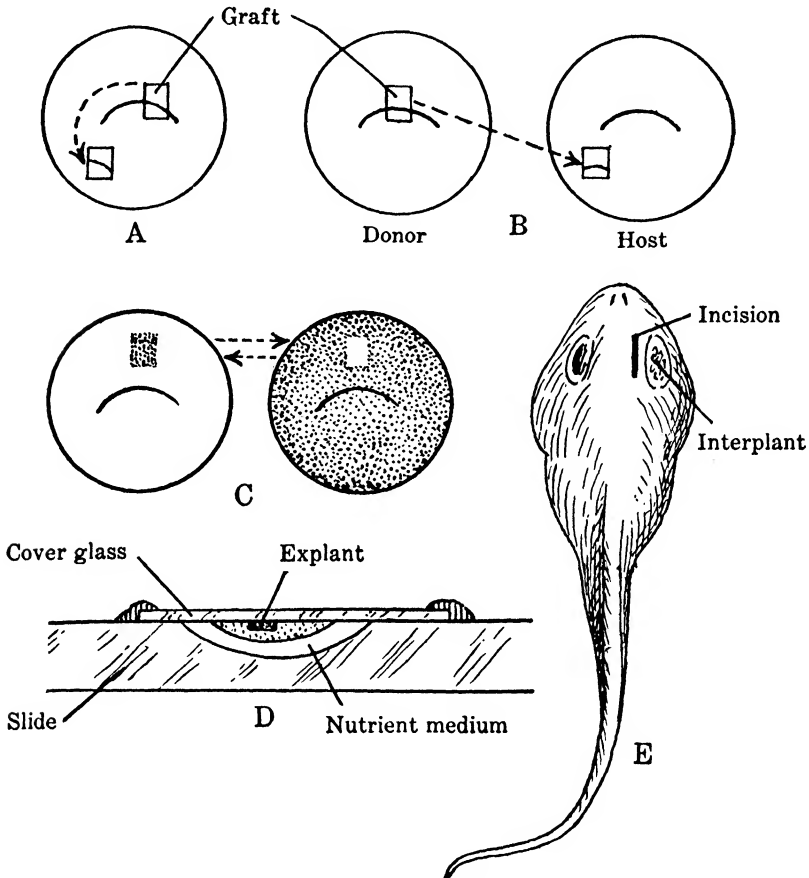


FIG. 109. — Diagrams to show different methods of transplantation, etc. A, homoplastic transplantation. B, heteroplastic transplantation (both donor and host of same species). C, xenoplastic transplantation (donor and host of different species). D, explantation (in vitro). E, interplantation. (Based on a diagram of Dürken.)

to the new locality. Thus, material which is presumptive epidermis can be transplanted into a region where it will become neural plate, mesoderm, or even endoderm. Or on the other hand, material which is presumptive endoderm can be made to develop into ectoderm or mesoderm by transplantation. The only exception to this rule is the region where the dorsal lip is to form.

This will never give rise to anything except dorsal lip and the structures arising from the dorsal lip. This exception will receive special attention later (page 169).

Very instructive experiments are those in which material is transferred from a species with heavy pigmentation (*Triton taeniatus*) to one with light pigmentation (*Triton cristatus*).

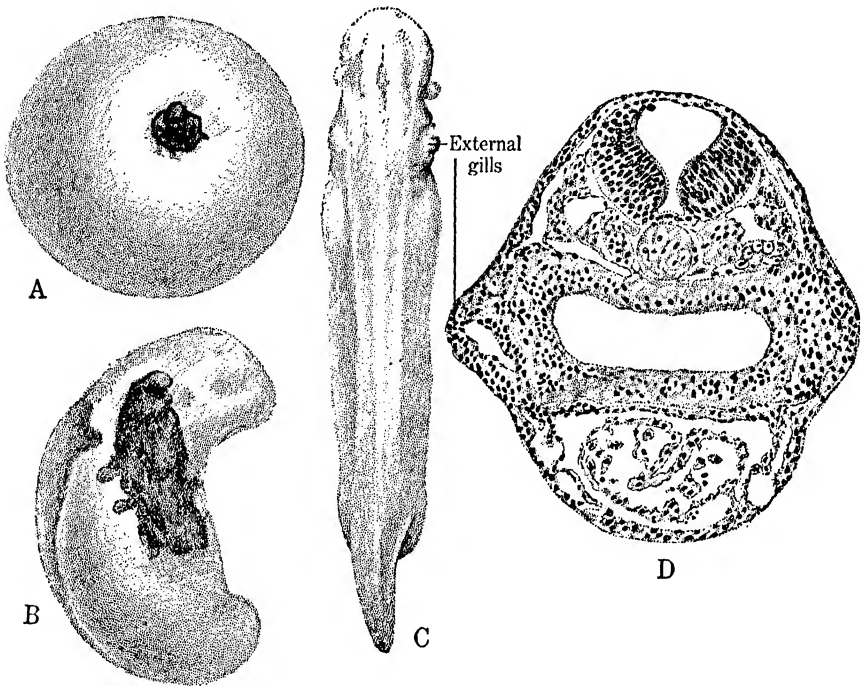


FIG. 110. — Xenoplastic transplantation between *Triton taeniatus* (dark), the donor, and *Triton cristatus* (light), the host to show early plasticity. A, immediately after transplantation. B, the transplant developing in the gill region. C, the gills of the transplant relatively more advanced. D, section through C in the gill region. (After Spemann.)

Here the graft preserves its racial character of pigmentation while otherwise conforming to the development of the host. Figure 110 illustrates such an example of xenoplastic transplantation. The light-colored graft from *T. cristatus* has developed into part of the neural tube of the host, where it stands out by reason of its light color. In the reciprocal transplantation (Fig. 111), the dark graft from *T. taeniatus* has given rise to the right external gills of the host.

The loss of plasticity (self-differentiation). — After gastrulation is well under way this plasticity seen in earlier stages is lost. The various regions of the embryo have become determined and

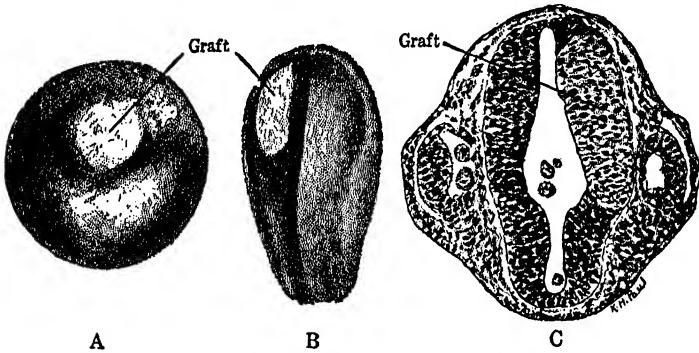


FIG. 111. — Reciprocal transplant to that shown in Fig. 110. Here *T. cristatus* (light) is the donor and *T. taeniatus* (dark) is the host. A, after transplantation. B, the transplant developing in the neural plate (region of the brain). C, section in later stage showing transplant developing in forebrain. (After Spemann.)

thenceforth will give rise only to the structures normally developing from them. In other words, the amphibian embryo does not undergo chemo-differentiation until this time. From now on it is a real mosaic. Figure 112 shows a neurula in which the various

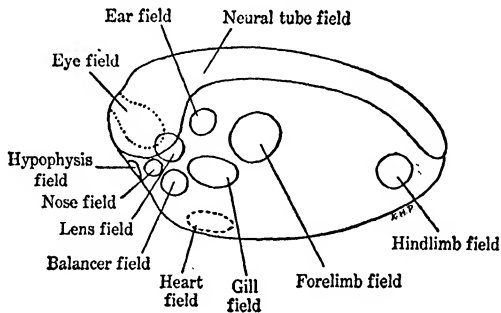


FIG. 112. — Diagram of an amphibia neurula showing organ fields as determined by transplantation experiments. (After Huxley and de Beer.)

organ fields are determined. If a bit of tissue is removed from the eye-field and transferred to the flank of another neurula (Fig. 113), it will give rise only to an eye, even in its new and abnormal environment.

Similar experiments have been carried on with the chick (implantation on chorio-allantois), and it has been proved that the

eye-field, ear-field, limb-buds, and other regions will develop and give rise only to the respective organs.

Very striking results have been obtained by implanting portions of rat embryos on the chorio-allantois of the chick, and a considerable amount of self-differentiation has been demonstrated.

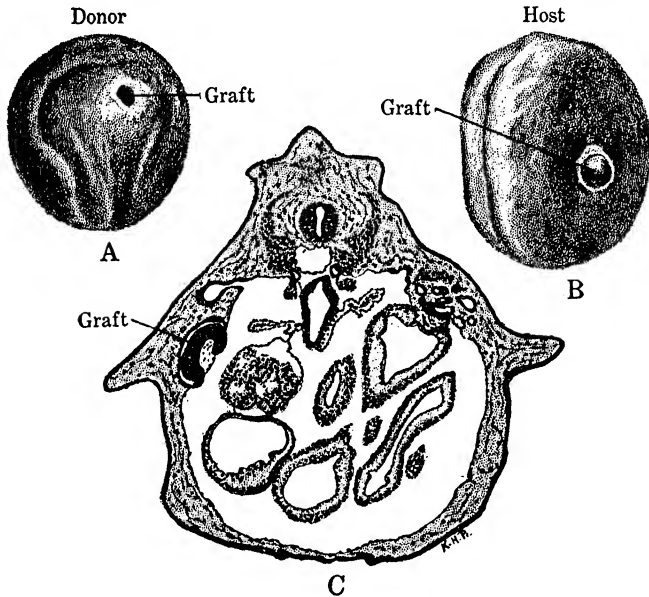


FIG. 113. — Self-differentiation in the toad *Bombinator*. A, donor in early neurula stage showing region from which graft was taken. B, host in late neurula stage. C, section through later embryo of host, showing graft forming optic cup in region normally occupied by pronephros. (After Spemann.)

The organizer. — The loss of its early plasticity by the embryo seems to be due to the presence of an organizer (organisator) as discovered by Spemann. In the amphibian embryo this is the dorsal-lip region, already mentioned. It will be recalled that this region alone of the presumptive organ fields of early gastrulation did not show the phenomenon of plasticity. Wherever it is transplanted it will become dorsal lip. But the most striking thing about this dorsal-lip region is that wherever it is transplanted it will bring about involution, and will transform the surrounding material into organ fields such as are ordinarily found about the dorsal lip. In a word, the grafted dorsal lip organizes a new,

secondary, embryo about itself, quite independent of the embryo which is organized about the dorsal lip of the host (Fig. 114).

The organizer itself undergoes involution beneath the surface of the host and becomes the notochord and the somite-mesoderm of the secondary embryo. The other structures, such as neural

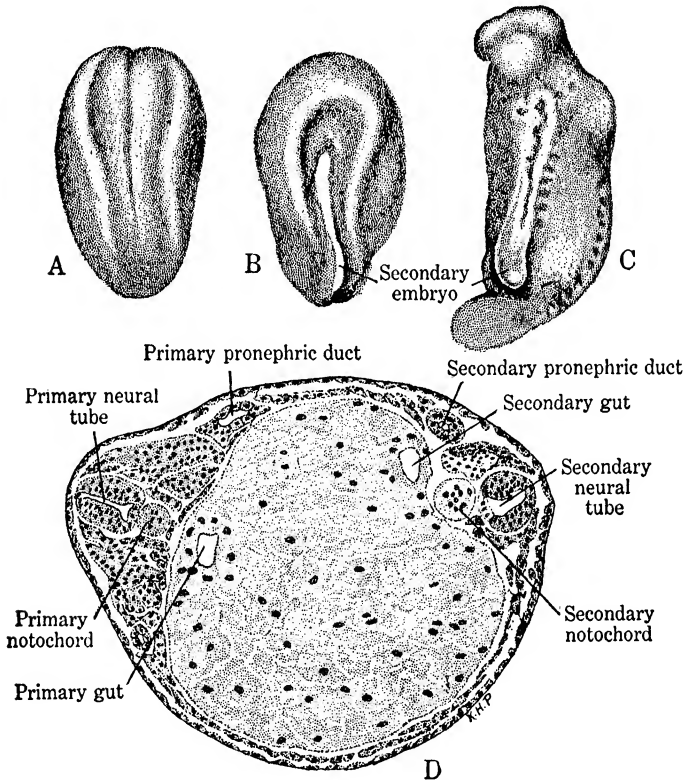


FIG. 114. — Effect of transplanting organizer. A, dorsal view of host (*Triton taeniatum*) in neurula stage. B, right side view at same stage showing secondary neural plate induced by organizer (dorsal lip region) of the donor (*Triton cristatus*) shown in white. C, later stage showing primary embryo in side view and secondary embryo in dorsal view. D, transverse section through C. (After Spemann and Mangold.)

plate, eyes, ears, kidney, heart, etc., arise from host tissue which has been brought under the influence of the organizer. Even after gastrulation this influence is continued, as can be shown by the following experiment. A bit of gastrocoel roof (notochord and mesoderm, in the urodeles), when transplanted into the

side of the gastrocoel, will induce the formation of neural folds above it.

So great are the powers of induction possessed by the organizer that it can cause presumptive ectoderm to become mesoderm or endoderm, and conversely, presumptive mesoderm can be transformed into ectoderm.

It is noteworthy that the organizer can exert its influence even in xenoplastic transplantation, e.g., the organizer from a toad can induce the formation of a secondary embryo in a newt. Apparently the effects of the organizer are physico-chemical in nature, for the dorsal-lip region can be narcotized, boiled, or even dried, and still induce the formation of a secondary embryo. It is suggestive that bits of agar after being in contact for some time with an organizer are themselves capable of producing induction. There is reason to believe that the glycogen (animal starch) content of the organizer has something to do with its effects, and quite recently, it has been reported that cephalin will bring out about the induction of a secondary embryo. Many parts of the adult vertebrate are capable of bringing about induction, but in the living embryo, the chemical substance responsible is found only in the organizer itself.

D. ENVIRONMENTAL FACTORS IN DEVELOPMENT

Many experiments have been carried on in the attempt to find the definite results produced on the developing embryo by changes in the environment. These investigations have established normal limits of temperature, etc., within which development can be completed. Within these limits, although development may be altered as to rate, etc., it is nevertheless carried on to a successful outcome. Beyond these limits the alterations are so profound as to produce monsters or cause death. Among the factors susceptible to experimental control are gravity, heat, light, the chemical constitution of the environment, and food.

Gravity (and centrifugal force). — It has been remarked (page 156) that the original polarity of the egg is not due to any effect of gravity. In telolecithal eggs, however, gravity may have some effect on the course of development. Thus frog's eggs when forcefully inverted may give rise to duplicate monsters. The hen's egg if not rotated at regular intervals fails to hatch. It has been

shown (Dareste, 1877) that this is due to the failure of the yolk sac to complete its development. It adheres to the allantois and cannot be retracted into the body as in normal development.

The influence of gravity may be shown in an exaggerated manner by prolonged centrifuging. It was found by O. Hertwig that, if the frog's egg is centrifuged during cleavage, the yolk is so

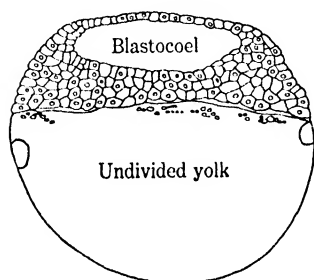


FIG. 115. — Vertical section through blastula of a frog's egg following centrifuging. (After Hertwig.)

concentrated in the vegetal hemisphere that the cleavage planes fail to cut through it and the end result is meroblastic cleavage suggestive of that seen in the chick (Fig. 115).

Heat. — The rate of development is directly affected by temperature. Thus for the egg of the frog (*Rana fusca*, Hertwig) the normal temperature is about 15°–16° C. From this point up to about 20°–22° C., development continues normally; beyond this limit it is abnormal, death ensuing

rapidly at 30° C. Below 15° C., development is retarded progressively with the drop in temperature, and at 0° C. cleavage ceases completely.

For the hen's egg, Kaestner determined the optimum temperature for normal development to be between 35° and 39° C. (95°–102° F.). The maximum temperature tolerated is 43° C., the minimum 28° C. (20°–21° C., Edwards).

Eggs of either frog or hen which have been exposed to extreme heat or cold and then returned to the optimum temperature often develop abnormally. A common type of monster is one in which neural plate and notochord are split (*spina bifida*).

Very striking results have been obtained by subjecting the eggs of the frog or the hen to a temperature gradient, that is, controlling the temperature so that one side is hotter or colder than the other. If the gradient runs along the polar axis, and the greater heat is applied to the animal pole, the result is that the embryos and larvae have overlarge heads; if the higher temperature is applied to the vegetal pole, the head region is subnormal. When the temperature gradient is applied laterally, the development of the heated side proceeds more rapidly than that of the cooled side.

It may be concluded that, within the limits of toleration, development is accelerated by increased temperatures and retarded by decreased temperatures.

Light and other forms of radiation. — In spite of a considerable number of experiments designed to determine the effects of definite intensities and wavelengths of light upon the developing embryo, the results are as yet too inchoate to be discussed in an elementary text.

Ultra-violet light, X-rays, and radium emanations in extreme dosage cause the cessation of development. In smaller dosage, they bring about anomalies (abnormalities in structure caused by disturbances in development). It should be remembered that the work of Müller and others indicates that these agents accelerate the rate of mutation of *Drosophila* genes, and so induce genetic point mutations as well as developmental anomalies.

Chemical composition. — The chemical composition of the surrounding medium affects profoundly the nature of development. The embryo cannot develop without oxygen, for it cannot live without respiration. It has been pointed out by Morgan that frog's eggs in the very center of the egg mass often develop abnormally (spina bifida, etc.). And it has long been known that the hen's egg ceases development if the pores of the shell are closed by water glass, varnish, or other agents.

Water, too, is an essential. The growth of the embryo depends upon the absorption of water, and all embryos must undergo their development within a watery medium. Even the terrestrial embryo has its private pond in the amnion. A slowing up in the rate of development, accompanied by abnormalities and a large percentage of deaths, results from incubating hen's eggs in a desiccator. The percentage of water in the frog's egg increases steadily during the first two weeks of development.

A very striking series of experiments was carried on by Herbst on the development of the sea urchin in artificial sea waters which had been made up omitting one after another of the elements found in normal sea water. Jenkinson, summarizing the evidence says:

“ The experiments which we have been considering are unique of their kind, and it is impossible to exaggerate their importance. For, whatever may be the ultimate explanation of the facts, there

can be no doubt whatever that the most complete demonstration has been given of the absolute necessity of many of the elements occurring in ordinary sea water, its normal environment, for the proper growth and differentiation of the larva of the sea urchin. Nor is this all. Some of the substances are necessary for one part or phase of development, some for another, some from the very beginning, others only later on. Thus potassium, magnesium, and a certain degree of alkalinity are essential for fertilization, chlorine and sodium for segmentation, calcium for the adequate cohesion of the blastomeres, potassium, calcium and the hydroxyl ion for securing the internal osmotic pressure necessary for growth, while without the sulph-ion and magnesium the due differentiation of the alimentary tract and the proper formation of the skeleton cannot occur; the secretion of pigment depends on the presence of some sulphate and alkalinity, the skeleton requires calcium carbonate, cilia will only beat in an alkaline medium containing potassium and magnesium, and muscles will only contract when potassium and calcium are there."

The addition of chemicals to the medium has resulted in many interesting disturbances in development. We can call attention here to two only. In the sea urchin it was found that the addition of lithium salts to sea water caused the embryo to undergo a very curious form of gastrulation, in which the endoderm and mesoderm were evaginated instead of being invaginated (Herbst). Such an embryo is called an exogastrula.

Quite recently, Holtfreter (1933) has induced exogastrulation in the egg of *Triton* by removing the egg envelopes and placing the developing egg in weak Ringer's salt solution. In the cases where development continued for some length of time (Fig. 116), it was discovered that the embryo developed in two parts, an ectodermal portion with no differentiation, connected by a narrow isthmus to a mesendodermal portion in which differentiation proceeded, but in an abnormal fashion. The embryo is inside-out. The mesendodermal portion of the exo-embryo develops a typical notochord, somites, kidney, gonad, a heart (empty), and a digestive tube, in which all the typical regions are indicated, including visceral pouches. These results confirm those of transplantation and explantation experiments discussed in an earlier section.

Food (including hormones and vitamins). — The amount and kind of food supplied to the developing young naturally affect the subsequent development. Thus, if frog tadpoles are fed on an exclusively vegetarian diet, the intestine becomes much longer than when an exclusively meat diet is offered. Specific foods often result in equally definite changes in the body. Thus

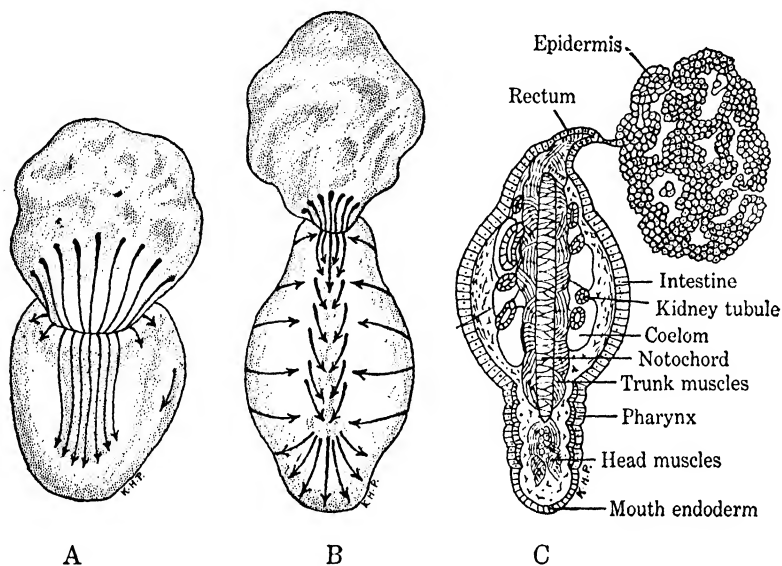


FIG. 116. — Exogastrulation in *Ambystoma*. A, B, exogastrulae showing direction of displacements during exogastrulation, compare Fig. 74. C, section of later exo-embryo. (After Holtfreter in Huxley and de Beer.)

Gudernatsch discovered that frog tadpoles fed on thyroid tissue grew less rapidly but underwent metamorphosis much more rapidly than the controls. Thymus-fed tadpoles, on the other hand, had a retarded metamorphosis accompanied by excessive growth. Later investigations indicate that the effects of thyroid are due to a hormone formed by this gland (thyroxin), which is a definite factor in bringing about amphibian metamorphosis.

It is interesting to note that by the use of thyroid or thyroxin the Mexican axolotl (Fig. 117) may be induced to undergo metamorphosis, when it becomes a normal *Ambystoma tigrinum*. Otherwise the axolotl becomes sexually mature in the larval condition (neoteny), and was, therefore, long thought to be a separate species.

In this connection we may refer briefly to the many experiments dealing with the effects of the various endocrine glands when given as food or as transplants and the effects produced when these glands are removed at their first appearance (extirpation). Without going into details, for the results of these experiments are sometimes ambiguous, we may say only that they

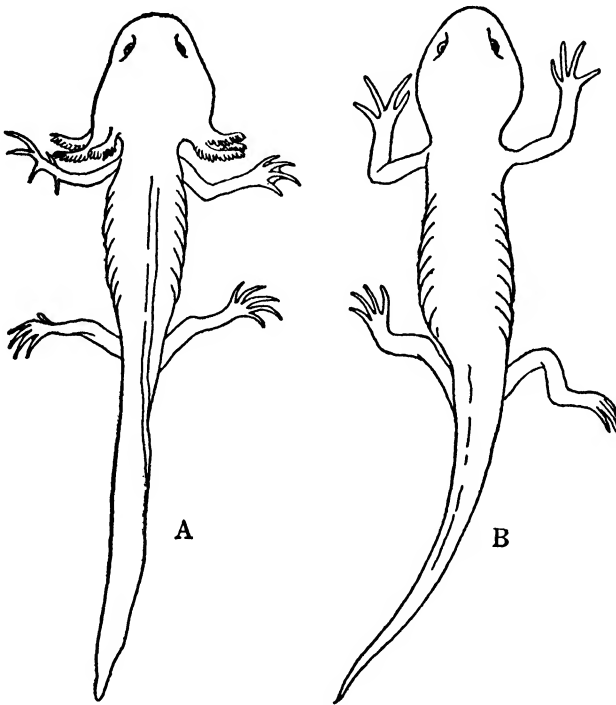


FIG. 117. — Metamorphosis in *Ambystoma*. A, neotenic larva (axolotl). B, metamorphosed adult. (After Dürken.)

indicate the importance of hormones in embryonic as well as in adult life.

The rôle of the vitamins in the metabolism of the embryo is too little understood at the present time for us to do more than allude to this subject. Vitamin E is often called the anti-sterility vitamin because its absence from the diet results in loss of the reproductive power. Adamstone (1931) in this laboratory has shown that the chick embryo produced by hens on a vitamin-E-free diet dies early in development following extensive disturbances in the blood-vascular system.

SUMMARY

Experimental embryology demonstrates that development is epigenetic. Given a suitable inheritance of genes, and a favorable environment, development proceeds normally through stages of increasing complexity. Any alteration, either in the genetic complex or in the factors of the environment, will bring about alterations in development.

The fertilized egg shows a definite organization as seen in its polarity and symmetry. These seem to be the expression of axial gradients. Sooner or later the cytoplasm of the egg undergoes chemo-differentiation and develops organ-forming substances — sooner in mosaic eggs, later in regulation eggs.

Cleavage segregates the organ-forming substances as they are differentiated, with the result that the isolated blastomeres of mosaic eggs have a limited potency, those of regulation eggs have a greater potency.

During germ-layer formation, the presumptive organ regions are segregated into the different germ layers. Among the vertebrates this reorganization is effected by an organizer, which in the frog is associated with the dorsal lip of the blastopore, and in the chick with the homologous primitive streak.

Even in regulation eggs a mosaic stage is established during germ-layer formation. The different organ fields are now determined, the earlier plasticity disappears, and each field is capable only of self-differentiation.

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PART III
ORGANOGENY

CHAPTER VIII

ENDODERMAL DERIVATIVES

The tissues derived directly from the endoderm are for the most part of the epithelial type and form the inner lining of the gastrocoel and the organs that arise therefrom. These organs are grouped into two closely connected organ systems, the digestive system and the respiratory system. The digestive (enteric) tube, however, becomes ensheathed in splanchnic mesoderm which contributes largely to the ultimate structure of the organ systems just mentioned. Furthermore, this tube opens to the exterior at both the anterior and posterior ends by means of two ectodermal pits, the stomodeum and proctodeum, respectively. All three germ layers, therefore, contribute to the organogeny of these systems.

The stomodeum. — There is an ectodermal invagination on the ventral side of the head to form the stomodeum (Fig. 118),

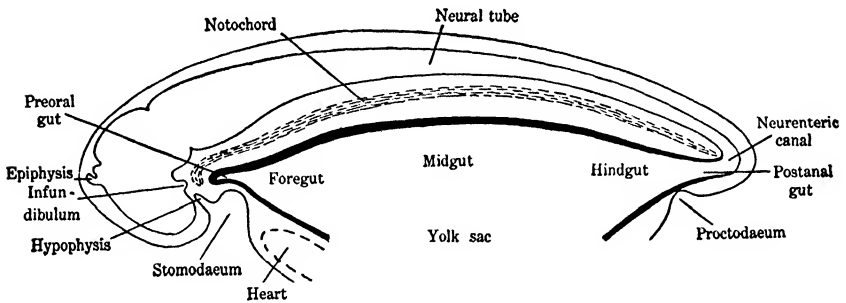


FIG. 118. — Diagram of an early vertebrate embryo, to show endodermal derivatives.

which is bounded on the sides by the maxillary ridges and on the rear by the mandibular ridges. The rupture of the oral plate, which separates the stomodeum from the fore-gut, results in the formation of the oral cavity, or mouth. From the stomodeum another invagination, the hypophysis, grows upward in front of the fore-gut, and eventually fuses with an evagination from the floor of the neural tube, the infundibulum, to form the pituitary

gland, an organ of internal secretion. As the stomodeum joins the fore-gut a little posterior to the anterior end of the latter cavity, there is a blind pocket of endoderm, anterior to the mouth, called the preoral gut.

The oral cavity. — The cavity of the mouth is a compound structure, derived in part from the ectodermal stomodeum and

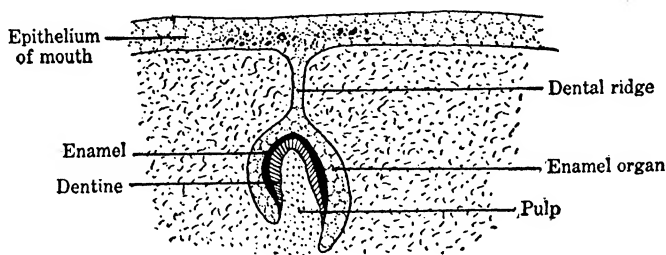


FIG. 119. — Diagram to show origin of vertebrate tooth (lower jaw).

in part from the endodermal fore-gut. The boundary line between these is soon lost after the rupture of the oral plate owing to unequal local growth of the different regions of the mouth. The boundaries of the mouth are the upper jaws, formed from the maxillary ridges, and the lower jaws, derived from the mandibular

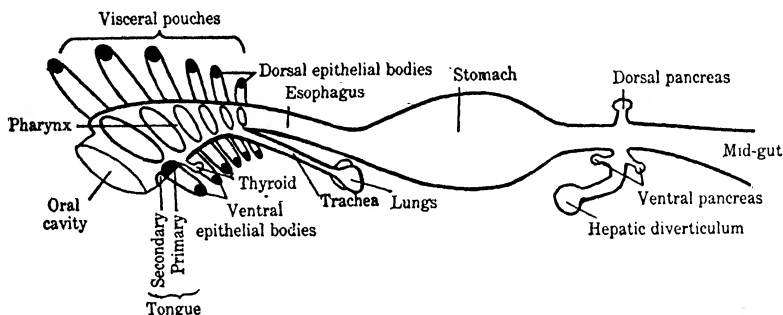


FIG. 120. — Diagram showing derivatives of vertebrate fore-gut.

ridges. On these ridges the teeth arise in exactly the same way as the placoid scales of the elasmobranchs (page 230). Two elements are concerned: an ectodermal enamel organ, shaped like an inverted cup; and a mesodermal dental papilla, which fills the cavity of the enamel organ. The enamel organ gives rise to the outer enamel layer of the tooth, while the papilla forms the dentine (Fig. 119). The dentine is in the general form of a hollow cone,

the cavity of which is filled with connective tissue, nerves, and blood vessels. The tongue (Fig. 120) is also a compound organ, arising from an endodermal primary tongue which is formed from the floor of the pharynx in the region of the hyoid arches, and from an ectodermal secondary tongue which arises from the floor of the oral cavity in front of the thyroid gland (page 184). Into the tongue a migration of mesoderm takes place, by means of which the musculature is formed. The glands of the mouth (salivary glands, etc.) arise from the ectodermal lining of the mouth. The taste buds, however, are endodermal (Holtfreter, 1933). The connection between the oral cavity and the nasal cavity will be discussed in Chapter X.

The pharynx. — The region of the fore-gut which follows the oral cavity is the pharynx, particularly important on account of the respiratory organs and other structures which arise from it.

Respiratory organs. — Respiratory exchange may take place in any thin epithelium in which the blood corpuscles are brought into contact with the oxygen-carrying medium. These epithelia may be either ectodermal or endodermal in origin. Thus, we find that among the amphibia, respiration may take place in the skin as a whole (lungless salamanders); in specialized outgrowths on the visceral arches, external gills (*Necturus*); or in the so-called "hairs" of the African frog, *Astylosternus*. In this group are to be found also examples of endodermal respiratory organs, the internal gills and lungs. Internal gills are otherwise found only among the fish, while the lungs are characteristic respiratory organs also of the amniotes.

The internal gills. — The internal gills (branchiae) arise in the visceral clefts (Fig. 120) common to all chordates. Among the aquatic vertebrates these are typically six in number (see Table 8, page 131). In the cartilage fish the first cleft (the spiracle) opens on the dorsal side of the head and is otherwise modified. The clefts are separated by the visceral arches, of which the first is known as the mandibular arch and the second is called the hyoid arch. The visceral clefts are formed by the coming together of paired evaginations of the endoderm (visceral pouches) and complementary invaginations of the ectoderm (visceral grooves). The ectoderm and endoderm come into direct connection to form closing plates. Later, these plates rupture and a series of finger-

like projections grow out into the cleft from the anterior and posterior sides of each arch. These filamentous processes usually fuse to form a demibranch (Fig. 197). The demibranchs in some fish are apparently of endodermal origin, while in the amphibia they are derived from the ectoderm. It is interesting to note that in the spiracle of the cartilage fish a gill-like structure, the pseudobranch, develops. In amphibians and the amniotes generally the first visceral pouch does not open to the exterior but gives rise to the tympanic cavity and auditory tube (see Chapter X). In all fish except the elasmobranchs, a projection grows back from the hyoid arch to cover the remaining visceral clefts. This is the operculum. Internal gills do not appear in the development of the amniotes; but the visceral clefts, or at least the visceral pouches and grooves, are of invariable occurrence.

The lungs. — In all the vertebrates except the cyclostomes and cartilage fish, there develops from the pharynx a sac (or a pair of sacs) which becomes the air bladder in pisces and the lungs in tetrapoda. We shall confine our attention here to the development of the lungs (Fig. 120). The first indication of lung formation is the appearance of a longitudinal groove in the floor of the pharynx posterior to the last pair of visceral pouches. This is the tracheal groove. This groove separates from the pharynx, the process commencing at the posterior end, so that the dorsal portion of the tube, or esophagus, is separated from the ventral portion, or trachea, except for a narrow opening, the glottis. The trachea grows backward rapidly and divides into two lobes, the primordia of the lungs. There is some evidence that the trachea is bifurcated from its first appearance, suggesting that the lungs arise from paired primordia. In the birds and mammals the lung primordia subdivide many times to form the bronchi, or branches of the respiratory tree.

The thyroid gland. — This structure arises as a median ventral evagination of the pharyngeal floor between the primary and the secondary tongue primordia or at the level of the hyoid arches. The diverticulum grows downward and expands at its distal end (Fig. 120). Eventually, its connection with the pharyngeal floor, the thyroglossal duct, becomes occluded and disappears, and the gland itself subdivides into a mass of vesicles which migrate backward and assume somewhat different positions in

various vertebrates, often ending as a paired organ on either side of the trachea.

The epithelial bodies.—In all the vertebrates there arise, from the upper or lower angles of the visceral pouches, small buds of epithelium which often give rise to endocrine glands of varying — and mostly unknown — function (Figs. 120, 121). The dorsal buds (except among the mammals, where conditions are reversed) contribute in varying number to the formation of a large gland, the thymus, which loses connection with the pharynx and moves backward to its definitive position, which differs according to the form studied. The remainder of the dorsal

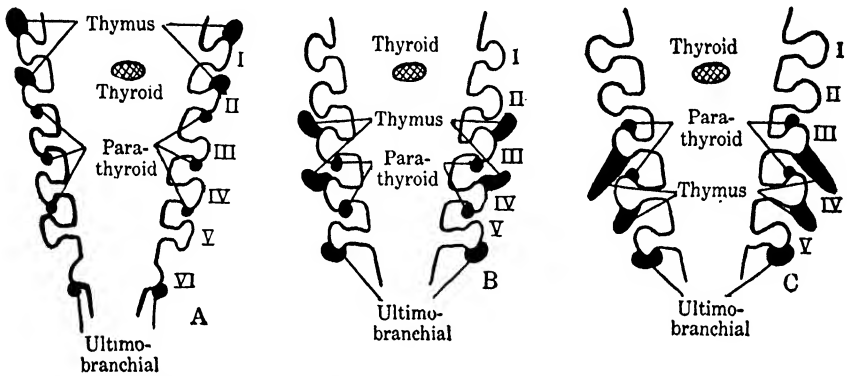


FIG. 121. — Diagrams showing origin of epithelial bodies in A, frog; B, chick; and C, man.

bodies become lymphoid and degenerate. The ventral buds (absent in fish) detach themselves from the pharyngeal wall and take up varying positions. Among the mammals it is the ventral buds which form the thymus, while the dorsal buds of the third and fourth pouches move to the sides of the thyroid gland where they are known as the parathyroids.

The esophagus.—The digestive canal behind the pharynx becomes specialized into four regions: (1) the esophagus; (2) the stomach; (3) the intestine and its derivatives; and (4) the cloaca. Of these, the esophagus (Fig. 120) remains comparatively unspecialized; it is a narrow tube, short in the anamniotes, elongate in the amniotes. No digestive glands are found in this region.

The stomach.—This portion of the digestive tract is distinguished by its dilation (Fig. 120) into a large sac or series of

sacs, and by the development of a thick wall of muscle from the splanchnic mesoderm in which it is enveloped. The stomach is rich in glands which aid in digesting the passing food.

The intestine. — All the regions of the digestive tract mentioned so far are derived from the fore-gut. The intestine is derived in part from the fore-gut, in part from the mid-gut, and in part from the hind-gut. It is impossible to indicate exactly which regions arise from these divisions of the gut, as both the fore-gut and the hind-gut expand at the expense of the mid-gut during the consumption of the yolk. As was said in the discussion of the development of body form, the division of the alimentary canal into these regions is the result of the method by which the head and tail are formed. The intestine becomes subdivided in various ways in the different groups, but we need notice only the most anterior of these, the duodenum, which is that portion of the intestine immediately succeeding the stomach and generally held to be derived from the fore-gut. The intestine is richly glandular throughout its length, but from the duodenum, in particular, we find developed two most important glands, the liver and the pancreas (Fig. 120).

The liver. — This gland arises from the ventral side of the duodenum as an evagination which grows forward, expanding into a vesicle at the distal end and retaining its connection with the duodenum by a narrow hollow stalk, the common bile duct, (Fig. 120). The sac-like distal end becomes subdivided, by the ingrowth of mesenchyme, into many tubules which often anastomose. In this process of growth and subdivision the liver grows about the vitelline veins (Chapter IX) and breaks these up into a system of hepatic capillaries. The cavity of the sac becomes the gall-bladder, to which the bile, formed in the glandular portion of the liver, is carried by means of the hepatic ducts. It releases these secretions into the duodenum *via* the common bile duct (ductus choledochus).

The pancreas. — This gland arises usually from three diverticula of the duodenum (Fig. 120), but the number of primordia is variable. One appears on the dorsal side of the duodenum just posterior to the stomach; the others arise on the ventral side, usually in connection with the hepatic diverticulum. The primordia increase in size, and break up into masses of secretory

tubules at the distal end of each. The primordia unite and their proximal ends become the pancreatic ducts, one or more of which may be suppressed in later organogeny. The pancreas, as well as elaborating a digestive pancreatic juice discharged through the pancreatic duct, forms a hormone (insulin), which is carried away by the blood stream. It functions therefore as an endocrine gland in addition to its digestive function. Insulin, as is well known, is important in the treatment of diabetes.

The cloaca. — The intestine behind the duodenum is variously subdivided in the different vertebrate classes, but all are alike in the possession of a terminal region which receives in addition the ends of the nephric ducts and of the genital ducts (see Chapter IX). From the cloaca also arises the urinary bladder and the allantois of the amniotes.

The cloaca, like the pharynx, communicates with the exterior by means of an aperture lined with ectoderm, which arises as a median ventral pit, the proctodeum (Fig. 118), just in front of the tail region. The proctodeum is formed at the point where the blastopore was obliterated and is separated from the hind-gut temporarily by means of the cloacal plate, which is comparable with the oral plate. For a time there is a blind pocket of endoderm posterior to the cloaca, which is known as the postcloacal gut. The region of the cloaca anterior to the entrance of the nephric ducts is known as the rectum; its aperture is called the vent. In mammals the rectum becomes separated from the remainder of the cloaca, which is then known as the urogenital sinus. Each of these cavities has a separate exit, the two openings being the anus and the urogenital aperture, respectively.

THE FROG (SEE ALSO CHAPTER XI). — The mouth of the tadpole does not open until a few days after hatching. It remains round during larval life and is enclosed by the mandibular ridges. Outside these, folds of ectoderm project as the larval lips, on which horny larval teeth develop. These larval structures are lost at metamorphosis, when the definitive jaws and teeth are formed in the usual way. The tongue is compound, arising from a primary tongue and a gland field, relatively late in larval life. The hypophysis is solid (Fig. 181).

Six visceral pouches appear, of which the first never becomes perforated, its closing plate becoming the tympanum of the ear,

and its cavity persisting as the tubo-tympanic cavity. Of the five remaining pouches, the second and third open to the exterior before the first and fourth, and the fifth remains vestigial. External gills appear on the third, fourth, and fifth arches (that on the fifth arch being rudimentary), but are resorbed later when covered by the operculum. This structure fuses with the body surface on the right side, but on the left it opens to the exterior by an opercular aperture. The internal gills appear as demibranchs commencing on the anterior side of the third arch. The first three gills, therefore, have two demibranchs, while the fourth has but one, formed from the anterior side of the sixth arch. The visceral clefts, gills, and opercular cavity are lost as separate structures by cell proliferation and reorganization just before metamorphosis. The lungs appear early in larval life as solid primordia of the pharynx. These acquire cavities prior to the formation of the tracheal groove which is relatively late in formation. The thyroid arises, just before hatching, as a solid diverticulum of the pharynx; it soon detaches itself and divides into two bodies which later become vesicular. The two thymus glands are formed from epithelial bodies on the dorsal side of the first and second visceral pouches. Epithelial bodies arise from the ventral sides of the second visceral pouches. It has been claimed that those of the third and fourth pouches become the carotid glands. The sixth pharyngeal pouches give rise to the ultimobranchial (suprapericardial) bodies. (Fig. 121A.)

The esophagus is short, and the stomach a simple dilation. The liver arises as a backward ventral diverticulum of the duodenum (Fig. 181). All three pancreatic primordia appear and fuse; the dorsal duct disappears, while the two ventral ducts fuse to become the adult pancreatic duct. The intestine of the tadpole, which is long and coiled (about nine times the body length), becomes resorbed during metamorphosis until it is about one-third of its larval length (Fig. 122).

The postcloacal gut loses its connection with the neural tube (neurenteric canal) during the backward growth of the tail. The urinary bladder does not appear until after metamorphosis.

THE CHICK (SEE ALSO CHAPTER XII). — The mouth opens on the third day of incubation. The teeth are represented only by the tooth ridges which are the first stage in the appearance of the

enamel organs. These appear on the sixth day of incubation and disappear shortly after the cornification of the jaws. This results in the formation of the beak and the egg tooth, the latter a horny projection on the upper jaw which is used in breaking through the shell at the time of hatching, and soon after disappears. The primordia of the tongue appear on the fourth day.

Five visceral pouches appear, of which the first three open to the exterior during the third day of incubation (Fig. 218). The

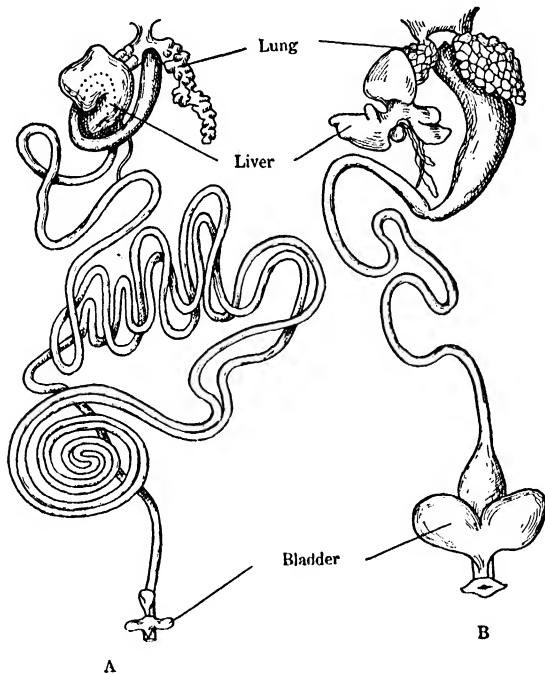


FIG. 122. — Digestive tube in A, tadpole, and B, frog, to show actual shortening of intestine. (After Leuckart wall-charts.)

first cleft closes during the fourth day, and the dorsal part of the pouch becomes the tubo-tympanic cavity. With the extension of the cervical flexure, the remaining pouches are crowded together and disappear. The thyroid appears on the second day, separates from the pharynx on the fourth, and on the seventh divides into two bodies which migrate backward to the junction of the common carotid and subclavian arteries. The thymus arises from the dorsal epithelial bodies of the third and fourth visceral pouches, while the parathyroid rudiments arise from

the ventral epithelial bodies. The fifth pouch gives rise to the ultimobranchial bodies. The lung primordia (Fig. 123) appear on the third day and grow back, becoming surrounded by mesenchyme. The primary bronchi subdivide to form a respiratory tree, some branches of which extend among the viscera and even into the hollow bones, as the accessory air sacs.

The esophagus is relatively long; and a dilation, the crop, forms at its posterior end. The stomach is divided into an anterior proventriculus, which contains the gastric glands, and a

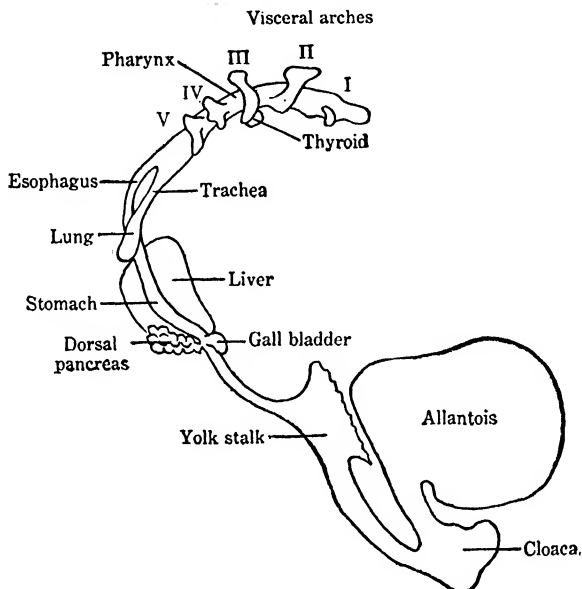


FIG. 123. — Endodermal derivatives in a 72-hour chick.

muscular gizzard at the posterior end. The liver primordium arises at the edge of the anterior intestinal portal on the second day and, therefore, presents the aspect of an anterior ventral and two posterior lateral diverticula for a short time. These fuse, however, by the end of that day, as the backward extension of the fore-gut continues. Three pancreatic diverticula are formed, the dorsal one on the third day, the ventral ones on the fourth. They fuse in later development, and either two or three of the ducts persist. The anterior portion of the mid-gut becomes the small intestine, the large intestine arising from the posterior region.

The cloaca is first distinguishable on the fourth day, when the proctodeum also is first apparent. The cloaca is ultimately divided into three regions: an anterior portion, the coprodeum, into which the rectum enters; an intermediate part, the urodeum, into which the nephric ducts and gonoducts enter; and the terminal proctodeum.

MAN (SEE ALSO CHAPTER XIII). — The mouth opens in the second or third week, and, like that of all vertebrates, develops lips (fifth week). Ten teeth papillae and enamel caps, the primordia of the milk teeth, appear in each jaw. This is a long-drawn-out process, the germs of the third molar not appearing until the fifth year of infancy. The tongue arises from swellings on the first three arches, the secondary tongue, or gland field, appearing as the tuberculum impar, which does not, however, appear to contribute to the ultimate structure of the tongue.

Five pairs of visceral pouches appear, none of which becomes perforated. The first gives rise to the tubo-tympanic cavity. The ventral portion of the second persists as the fossa in which the tonsil develops. The dorsal epithelial bodies from the third and fourth pair of pouches become the parathyroids. The ventral epithelial bodies of the third pair of pouches unite to form the thymus gland. Similar bodies from the fourth pair may give rise to vestigial thymus-like bodies which remain attached to the parathyroids from the same pouch. The fifth pair become the ultimobranchial bodies. The thyroid gland undergoes an incomplete division into two lobes which remain connected by a narrow isthmus. The lungs (Fig. 124) arise toward the end of the fourth week, from a laryngo-tracheal groove. The cartilages and musculature of the larynx arise from the branchial arches.

The esophagus, at first relatively short, lengthens as the backward movement of the heart and lungs displaces the stomach. The latter organ arises as a dilation of the fore-gut posterior to the esophagus. Continued growth, mainly on the dorsal surface, produces the greater curvature, and a displacement of the whole organ so that the cephalic end is moved to the left and the caudal end to the right. This is followed by a rotation of the stomach on its long axis through 90° to the left. The liver arises during the third week as a ventral groove in the duodenum. The pancreas appears slightly later, with either two or three

primordia according to whether or not one of the ventral primordia is suppressed. The ventral pancreatic duct persists and opens into the common bile duct. The point of division between small and large intestines is marked by the formation of a blind pouch,

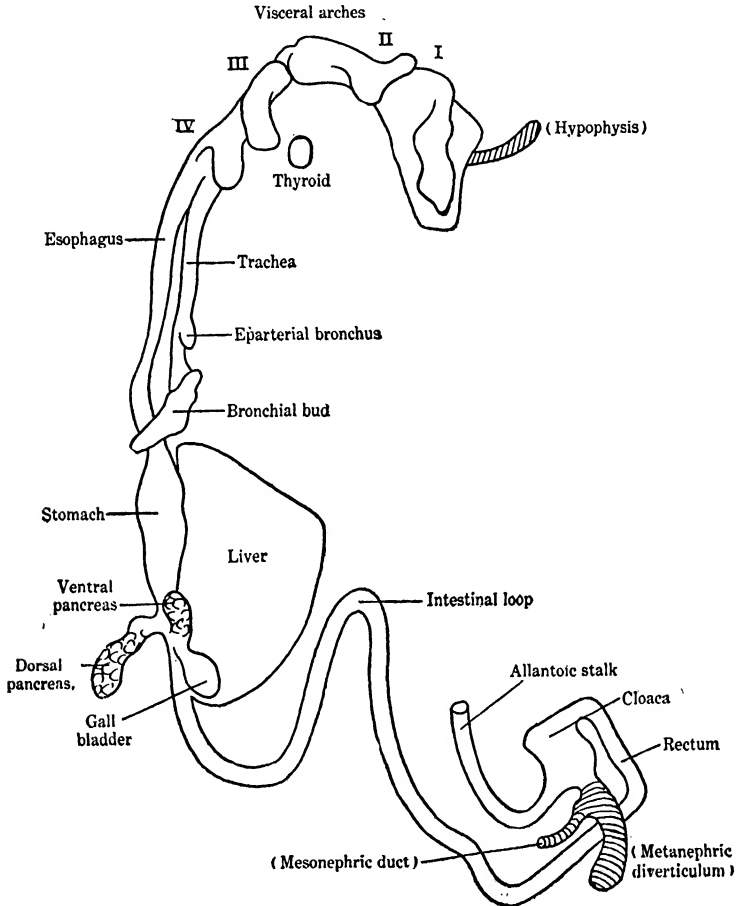


FIG. 124. — Endodermal derivatives in 10-mm. pig. (From a wax reconstruction by G. W. Hunter and L. T. Brown.)

the cecum. The distal end of the cecum does not grow as rapidly as the proximal region and so remains a finger-like projection known as the vermiform appendix. The small intestine, growing more rapidly than the large, is thrown into a set of six primary coils, each of which develops secondary coils.

The cloaca becomes divided, by a frontal partition, into a

dorsal rectum and a ventral urogenital sinus. The cloacal membrane is correspondingly divided into a rectal and a urogenital plate, and the final openings are the anus and the urogenital aperture. The urogenital sinus later is divided into a phallic portion (see page 211) and a vesico-urethral portion. The latter gives rise to the urinary bladder at its distal end, and to the urethra at its proximal end.

SUMMARY

The endoderm gives rise to the epithelial lining of the following structures:

A. Fore-gut

I. Oral cavity (also partly from ectoderm of stomodeum)

Teeth (also partly from ectoderm)

Tongue

II. Pharynx

Trachea and lungs

Thyroid

Visceral pouches

Auditory tube and chamber

Fossa of palatine tonsil

Thymus

Parathyroids

Ultimobranchial bodies

III. Esophagus

IV. Stomach

V. Duodenum

Liver

Pancreas

B. Mid-gut

I. Intestine

C. Hind-gut

I. Cloaca (also partly from ectoderm of proctodeum)

Rectum

Urogenital sinus

Urinary bladder

Urethra (also partly from mesoderm, page

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CHAPTER IX

MESODERMAL DERIVATIVES

The middle germ layer arises as three different aggregates of cells between the ectoderm and endoderm: the notochord; the mesoderm; and the mesenchyme. The origin of the notochord has already been described, and its later history will be discussed in connection with the skeleton. Organs of mesenchymatous origin will be taken up in connection with the history of the region from which their mesenchyme originates. Of the structures derived from the mesoderm, we shall consider first those arising from the lateral mesoderm, then those whose origin is from the intermediate mesoderm, and finally those derived from the axial mesoderm.

A. THE COELOM AND ITS MESENTERIES

Cavities may appear in all three divisions of the mesoderm; if in the myotomes, they are known as myocoels; if in the nephrotomes, they are called nephrocoels; the cavity of the lateral mesoderm is the coelom (Fig. 76). In some forms the three cavities are confluent. The connection, however, is a temporary one, and the myocoels soon disappear. In other forms they make a transitory appearance and are entirely disconnected with the other cavities, and in many vertebrates myocoels are never formed. The nephrocoels will be considered with the nephric organs. The coelom in *amphioxus* has a metameric origin from the ventral portions of the enterocoels, which become confluent at this point by the disappearance of the intervening anterior and posterior partitions. In vertebrates the coelomic cavity arises from the splitting of the lateral mesoderm into a dorsal somatic and a ventral splanchnic layer. In the amniotes this split continues out into the extra-embryonic mesoderm, thus giving rise to the exocoel, or cavity of the chorion. The coelom does not extend anterior to the visceral arches. Transitory cavities have been found in the arches and, indeed, in the head

itself, and these have been interpreted as the remains of a cephalic coelom. It will appear later that these are more probably the rudiments of cephalic myotomes. The coelom does not extend into the tail.

Somatopleure and splanchnopleure. — The somatopleure has already been defined as the outer layer of the lateral mesoderm together with the ectoderm with which it becomes associated. Between these two there is an invasion of mesenchymatous cells from the dermatomes and myotomes which give rise to the corium of the skin (see Chapter X) and to its dermal musculature (see page 239). The somatic mesoderm lining the outer wall of the coelom becomes the outer peritoneal lining. The splanchnopleure is the inner layer of the lateral mesoderm plus the endoderm with which it is associated. Between these two occurs a migration of mesenchyme cells which give rise to the splanchnic musculature and blood vessels, while the splanchnic mesoderm itself forms the inner peritoneal lining of the coelom.

The mesenteries (Fig. 125). — In all the vertebrates, the coelom is divided for a time into right and left halves by sagittal partitions above and below the alimentary canal, known as the dorsal mesentery and the ventral mesentery, respectively. These are formed by the inward growth of the splanchnic mesoderm above and below the digestive tube and the subsequent fusion of these sheets in the median line. The ventral mesentery disappears posterior to the liver, probably in connection with the coiling of the intestine. The dorsal mesentery (Fig. 125) persists as the support of the alimentary canal, and frequently becomes subdivided into regions which are named from the supported organ, such as the mesogastrium which supports the stomach, the mesoduodenum, etc. In the formation of the ventral mesentery, two organs, the heart and the liver, owing to their ventral position, are caught in between the two advancing sheets of splanchnic mesoderm. In these regions, therefore, the ventral mesentery is divided into an upper and a lower half. The ventral mesentery dorsal to the heart becomes the dorsal mesocardium; that part which is ventral to the heart is the ventral mesocardium (Fig. 126A). Both eventually disappear as the heart increases in size and complexity. In the region of the liver, the dorsal half of the mesentery becomes the dorsal mesohepar, while the ventral por-

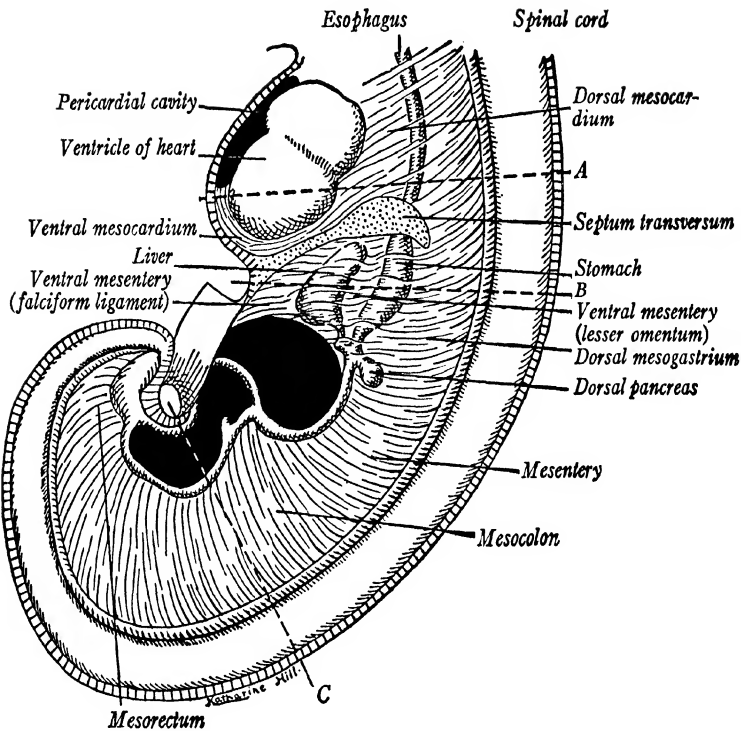


FIG. 125. — Diagram of mesenteries in early human embryo from left side. A, B, and C indicate planes of sections shown in Fig. 126. (From Arey after Prentiss.)

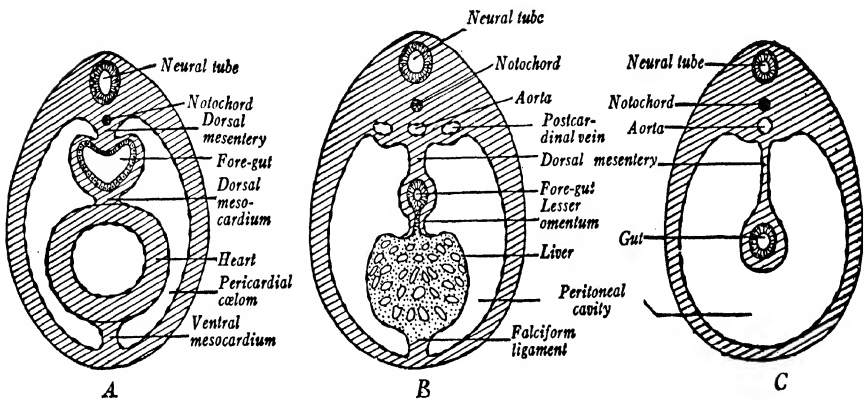


FIG. 126. — Diagrams of mesenteries in early human embryo as seen in transverse sections. Compare Fig. 125. (From Arey after Prentiss.)

tion is the ventral mesohepar (Fig. 126B). The primordia of the pancreas lie originally in the dorsal and ventral mesenteries, respectively, but with the rotation of the stomach all are included in the dorsal mesentery. The peritoneal supports of the nephric and genital organs will be considered in the following section. The spleen (see page 224) arises in the mesogastrium, close to the wall of the alimentary canal, and is probably mesodermal in origin.

Later divisions of the coelom. — The coelom becomes divided into an anterior pericardial cavity surrounding the heart, and a posterior abdominal cavity surrounding the viscera, by the septum transversum, a transverse partition which grows out from the bridge of mesoderm surrounding the vitelline veins

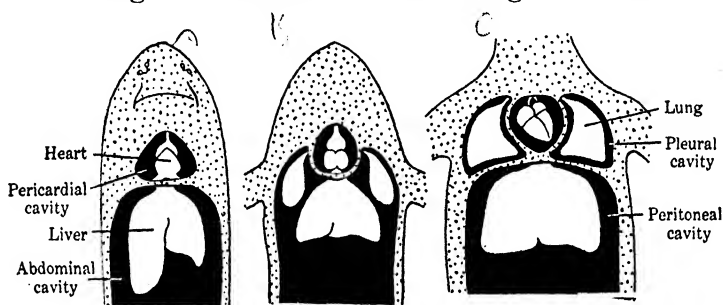


FIG. 127. — Diagrams of coelom and its divisions in A, fish, B, amphibia, reptiles and birds, and C, mammals. (After Kingsley.)

where they cross the coelom *en route* from the body wall to the heart (Fig. 127A). These cavities are connected during a large part of the embryonic period by pericardio-peritoneal canals where the septum has failed to unite with the ventral body wall. In the amniotes, additional septa develop behind the lungs and separate the pleural cavities, which contain the lungs, from the remainder of the abdominal cavity, which is now known as the peritoneal cavity (Fig. 127B). The pleural cavities are separated from each other in the median line by the mediastinum. In the mammals (Fig. 127C) the partition separating the lungs from the viscera receives musculature from the myotomes and becomes the diaphragm.

THE FROG (SEE ALSO CHAPTER XI.) — In the frog, the ventral mesentery disappears as soon as it has been formed, except in the region of the heart and liver. The ventral mesocardium appears

before the dorsal mesocardium is formed, and disappears soon after, to be followed by the disappearance of the dorsal mesocardium. The ventral mesohepar also has but a short period of existence. The septum transversum receives much of its substance from the mesodermal sheath of the liver. No pleural cavities are formed.

THE CHICK (SEE ALSO CHAPTER XII.)—In the chick, both dorsal and ventral mesenteries are formed. The latter, however, persists only in the region of the fore-gut, and gives rise to the mesocardia, which soon disappear; the dorsal mesohepar, which becomes the gastro-hepatic omentum, and the ventral mesohepar, which becomes the falciform ligament. The septum transversum is not completed until the eighth day of incubation. The pleural cavities are cut off from the pericardial cavities by a pleuro-pericardial septum, and from the peritoneal cavity by the pleuro-peritoneal septum.

MAN (SEE ALSO CHAPTER XIII.)—From the first, the pericardial cavity is distinguishable from the abdominal cavity, inasmuch as it never communicates directly with the extra-embryonic coelom as does the abdominal cavity. As in the chick, its posterior boundary is coterminous with that of the fore-gut, but it is in communication with the abdominal cavity by means of the parietal recesses, passages which correspond to the peritoneo-pericardial canals of the anamniotes. The recesses are divided frontally by the vitelline veins into dorsal and ventral parietal recesses. With the formation of the septum transversum, the ventral recesses are incorporated into the pericardial cavity. The dorsal recesses become the pleural cavities; and the pleuro-peritoneal septum, which divides them from the peritoneal cavity, is formed by the upward growth of the diaphragm. The musculature of this organ arises from the fourth cervical myotome during the backward growth of the diaphragm. The rotation of the stomach results in a rearrangement of the mesenteries, for an account of which the reader is referred to Hertwig or Keibel and Mall.

B. THE NEPHRIC ORGANS

The nephric or excretory system of vertebrates is essentially a paired series of tubes (nephridia), developed in the intermediate mesoderm, which collect nitrogenous wastes from the blood and

discharge them to the exterior by two longitudinal ducts emptying into the cloaca. The intermediate mesoderm in the anterior

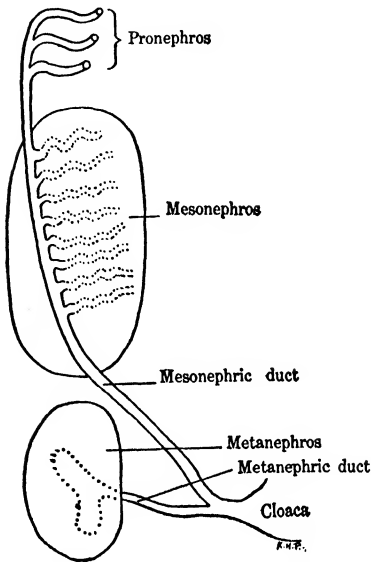


FIG. 128. — Diagram to show relationships of vertebrate excretory systems.

part of the body is divided into nephrotomes corresponding to the somites. There are three different types of kidneys among the vertebrates (Fig. 128). The first is the pronephros, which arises from the anterior nephrotomes and is the functional kidney in the larval stages of the fish and the amphibians. The second is the mesonephros, which arises from nephrotomes posterior to the pronephros and is the functional kidney of adult anamniotes and embryonic or fetal amniotes. The third is the metanephros which is the functional kidney of adult amniotes.

The pronephros. — This organ is formed during development by all vertebrates, but is best developed

in larval types like the frog, where it arises from nephrocoels (Fig. 129) in the anterior nephrotomes (III, IV, V), the dorsal ends of which grow caudally and unite with each other to form the pronephric duct which grows backward toward the cloaca. The ventral ends of the nephrocoels open into the coelom, and these openings, the nephrostomes, become lined with long cilia. The tubules meantime elongate and become contorted as they project into the surrounding posterior cardinal vein. Median to each nephrostome, the splanchnic mesoderm bulges out and in this projection develops a net of capillaries, or glomerulus, which becomes connected with the dorsal aorta. The pronephros is functional, at most, for a short time; and it disappears as the mesonephros develops to replace it.

The mesonephros. — The mesonephros, like the pronephros, is developed by all vertebrates. It becomes the adult kidney of the anamniotes, but is functional during the embryonic (and fetal) period only of the amniotes. Portions of the mesonephros

become associated with the genital organs of the adult (see next section).

The mesonephros also develops as a series of segmental nephrocoels, but in the nephrotomes posterior to those containing the

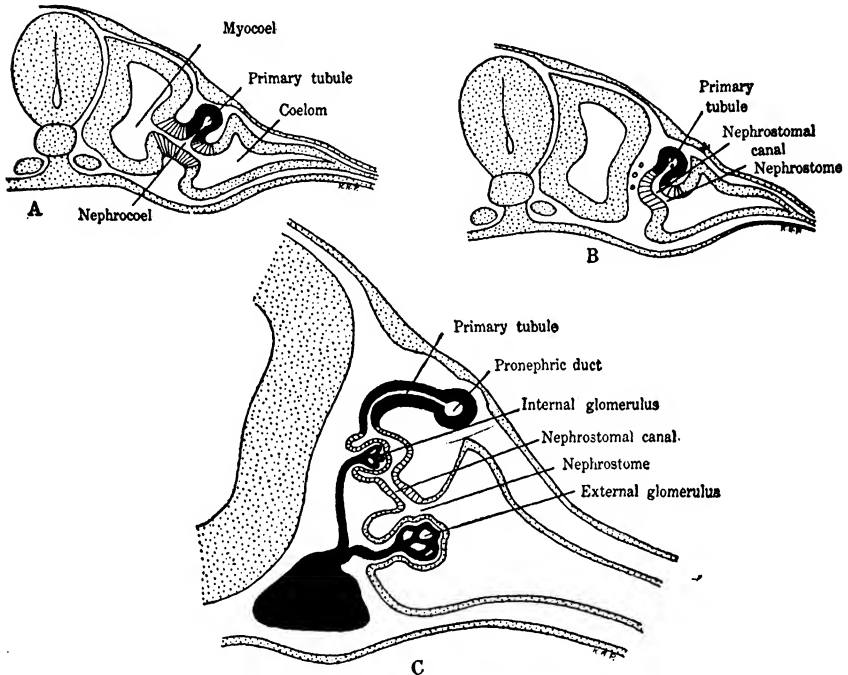


FIG. 129. — Diagrams showing three stages in the development of the pronephric tubule. (After Felix.)

pronephric ducts with which they unite (Fig. 130). After the degeneration of the pronephros, the tube is known as the mesonephric or Wolffian duct. The ventral ends of the nephrocoels acquire coelomic nephrostomes in the anamniotes. In amniote development, nephrostomes are seldom formed. A glomerulus connected with the dorsal aorta and the cardinal veins arises in connection with each tubule, as in the pronephros. An important difference between the pronephros and the mesonephros lies in the fact that the number of nephric tubules in each nephrotome is greater in the mesonephros (Fig. 131). These arise by the constriction of the posterior median part of each nephrocoel into a small vesicle which gives rise to a secondary tubule; each of these tubules acquires a glomerulus and nephrostome at the

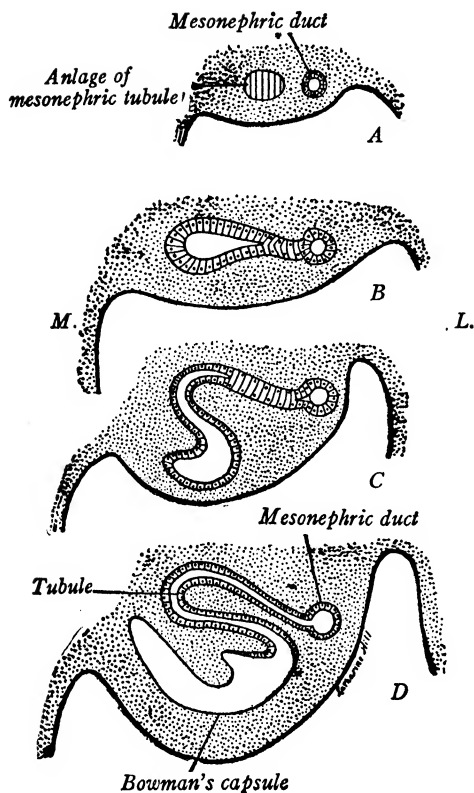


FIG. 130. — Diagrams showing four stages in development of mesonephric tubule. (From Arey after Felix.)

the metanephros has been formed. ✓

The metanephros.

— The metanephros, which is found as a separate kidney only in adult amniotes, probably is equivalent to the posterior portion of the mesonephros of the anamniotes, which it resembles greatly in its organogeny.

proximal end. The connection of these secondary tubules with the Wolffian duct, however, is attained by an evagination from the duct itself which grows out as the collecting duct to meet the developing secondary tubule. From these secondary tubules, tertiary ones bud off and develop in like manner, acquiring connections with the collecting duct through an evagination of this canal. As many as eight tubules may be formed in a single segment by this process of budding. This complexity is greatest at the posterior end of the mesonephros. In the amniotes, the mesonephros (except for that portion associated with the genital organs) disappears after

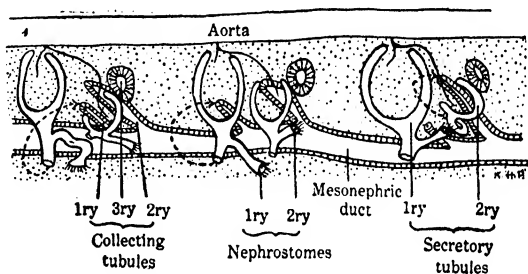


FIG. 131. — Diagram to show origin of secondary and tertiary mesonephric tubules from primary tubules. (After Brauer.)

The region in which the metanephros arises is, like that in which the earlier kidneys are found, the intermediate mesoderm. But in the posterior region of the body this mass is never segmented into separate nephrotomes. The first indication of metanephros formation is the appearance of an evagination from the dorsal surface of the mesonephric duct near the point at which the latter enters the cloaca. This evagination grows dorsally and

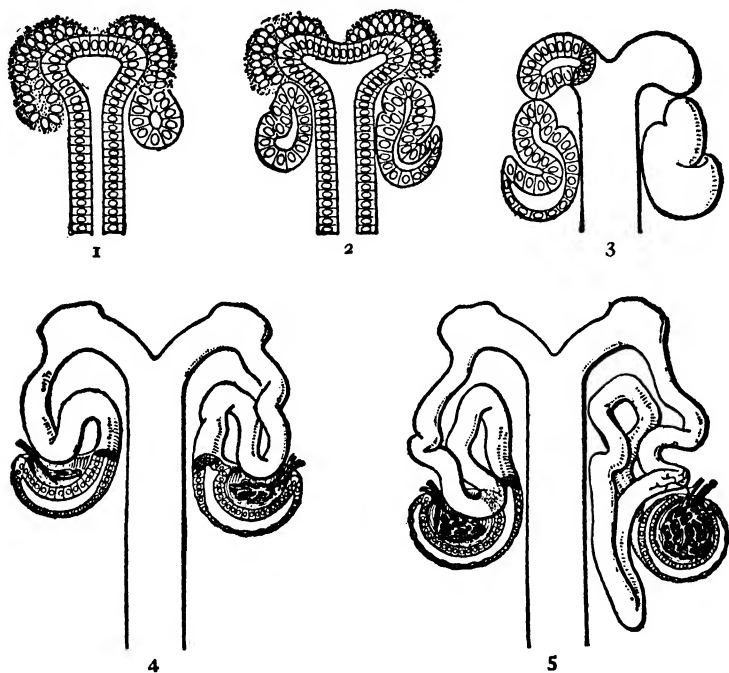


FIG. 132. — Diagrams to show origin and development of metanephric tubules. Collecting tubule in center, secretory tubules to right and left, the one on the right relatively more advanced. (From Arey after Huber.)

then turns forward to become the metanephric duct, or ureter, in much the same manner as the collecting ducts of the mesonephros arose. The metanephric duct then sends out into the nephrogenous tissue evaginations which increase in length and branch repeatedly to form the collecting tubules of the metanephros. Around the distal end of each tubule, a small mass of the nephrogenous tissue condenses and acquires a lumen like the nephrocoels of the pronephros and mesonephros (Fig. 132; 1, 2). From these vesicles the secretory nephric tubules arise by a process of elonga-

tion and later fuse with the collecting tubules just described (Fig. 132; 3, 4). In each of the tubules a capsule develops for the reception of a glomerulus which later acquires a connection with a branch of the renal artery (Fig. 132; 4, 5). Development proceeds from the posterior end toward the anterior, instead of in the opposite direction as in the earlier types of kidneys. The portion of the Wolffian duct nearest to the cloaca is absorbed by it so that the ureter has an opening into the cloaca separate from that of the mesonephric duct. From the region of the cloaca into which the ureters open is formed the urinary bladder and urethra (page 193). In mammals, at least, the enlarging bladder includes part of the ureter.

The later history of the kidneys and their ducts is considered in the next section.

THE FROG (SEE ALSO CHAPTER XI). — Three pronephric tubules are formed (somites II, III, IV), each with a nephrostome. The region of the coelom into which these open is cut off ventrally by the development of the lungs and becomes the pronephric chamber. The glomeruli soon unite to form a glomus. Before metamorphosis the pronephric tubes, and that portion of the duct into which they open, degenerate.

Mesonephric tubules appear in the nephrotomes (somites VII–XII). These have nephrostomes in early larval life; but at the time the pronephroi degenerate the portion of each mesonephric tubule next to the nephrostome (peritoneal canal) breaks away from the remainder of the tubule and fuses with the posterior cardinal vein. The mesonephros is the functional kidney of the adult, and the Wolffian duct, therefore, functions as the ureter.

THE CHICK (SEE ALSO CHAPTER XII). — About twelve pronephric tubules arise (somites V–XVI) beginning on the second day of incubation. Nephrostomes are formed, but glomeruli do not appear until the third and fourth days of incubation, at which time the pronephros is degenerating. The pronephric duct arises at the ninth somite.

Mesonephric tubules arise from the intermediate mesoderm between somites XII and XXX, the more anterior of which develop nephrostomes. The main part of the mesonephros, however, arises between somites XX and XXX, where the continued growth of the tubules causes the projection of this region

into the coelom as the Wolffian body. It is extremely doubtful whether the mesonephros ever functions as a kidney, as it begins to degenerate on the eleventh day.

The metanephros arises on the fourth day of incubation, from two primordia as usual, the intermediate mesoderm in somites XXXI-XXXIII, and an evagination of the mesonephric duct, comparable to the collecting ducts of the mesonephric tubules, which becomes the ureter, pelvis, and collecting ducts.

MAN (SEE ALSO CHAPTER XIII). — Pronephric tubules arise in somites VII-XIII, develop nephrostomes and glomeruli, but degenerate rapidly.

Mesonephric tubules appear in the intermediate mesoderm between the sixth cervical and fourth lumbar segments, but those of the cervical and thoracic segments soon degenerate. Nephrostomes are formed by the more anterior tubules but have only a transitory existence. The mesonephros does not function as a kidney.

The metanephros has a double origin as in the chick.

C. THE GENITAL ORGANS

The genital organs may be grouped into two classes: (1) the primary genital organs, or gonads, in which the germ cells develop; and (2) the accessory genital organs, whose original function is the discharge of the germ cells from the body.

The gonads consist of the germ cells and the subordinate tissues, blood vessels, nerves, connective tissue, etc., which make up a large part of these glands. In an earlier chapter it has been shown that the primordial germ cells may first appear in the endoderm of the gut wall and thence migrate by way of the splanchnic mesoderm, dorsal mesentery, and peritoneum to their definitive position in a thickening of the peritoneum on the mesial side of the nephrotomes. This thickening is called the genital ridge (Fig. 133B). A considerable body of evidence is accumulating to indicate that germ cells may also arise from the cells of the genital ridge itself.

The genital ridge is now invaded by mesenchymal cells, and projects into the coelomic cavity. In some amphibians, a metameric arrangement corresponding to the myotomes has been recorded, but following this the segments unite. The anterior

and posterior ends of the ridge degenerate, and the middle portion enlarges and is separated by a longitudinal groove from the mesonephros so that it hangs in the coelom suspended by a fold of the peritoneum, known as the mesorchium in the male or the mesovarium in the female. The germ cells have by this time become transformed into gonia (Chapter III) and the germ glands are known as gonads.

Within the gonads, the gonia come to lie in nests, close to the germinal epithelium. Tubular outgrowths from the nephric

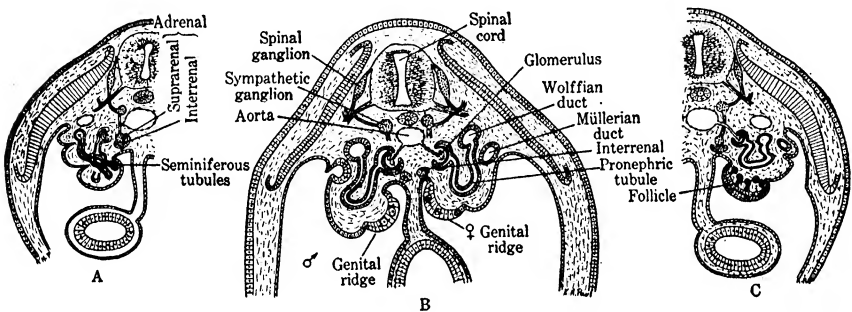


FIG. 133. — Diagrams to show early development of the gonads in transverse sections. A, testis. B, genital ridge. C, ovary. (After Corning.)

tubules of the mesonephros approach these nests. The later history of the gonads differs in the two sexes.

Testis. — In the male, the nests of spermatogonia become tubules which connect with the tubules growing in from the mesonephros (Fig. 133A). The testicular parts of these canals are known as the seminiferous tubules, the nephric portions as the efferent ductules. The walls of the seminiferous tubules are composed of spermatogonia and sustentacular cells which act as nurse cells to the developing sperm. Between the tubules lie partitions of mesenchyme which make up the stroma of the testis and contain the interstitial cells, which are supposed to be concerned in the formation of the male hormone. It is because of the presence of these cells that the testis is sometimes spoken of as the “interstitial gland.” It is now well established that the testis secretes a “male” hormone whose presence in the blood has much to do with the male secondary characters. Eventually, the tubules become separated from the surrounding germinal

epithelium by the development of a mesenchymatous layer called the tunica albuginea.

Ovary. — In the female, the nests of oögonia become separate follicles (Fig. 133C) which never attain connection with the mesonephric tubules. These tubules consequently degenerate. A follicle consists of a single oögonium surrounded by follicle cells which may be compared to the sustentacular cells of the male. In the mammalian ovary the primary follicle is greatly enlarged to form a vesicular (Graafian) follicle (Fig. 134), which protrudes

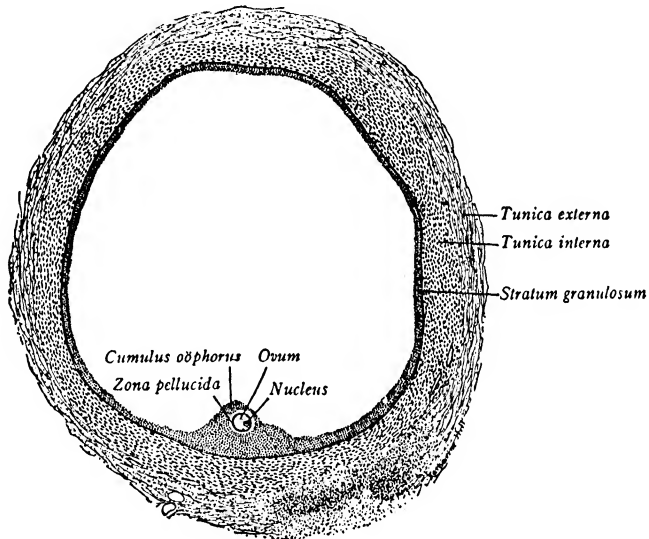


FIG. 134. — Section of human vesicular (Graafian) follicle. (From Arey after Prentiss.)

from the surface of the ovary. The follicle cells multiply and secrete a follicular fluid which presses the outer wall (stratum granulosum) away from the egg and a layer of follicle cells immediately surrounding it. These form a projection (cumulus oöphorus) into the cavity of the follicle. When ovulation takes place the wall of the follicle is ruptured, and the egg, still surrounded by its investment of follicle cells, now known as the corona radiata (page 41), is washed out with the follicular fluid. After ovulation the follicle cells enlarge, multiply, and secrete a yellow substance, the whole forming a corpus luteum. Hisaw has identified hormones from corpus luteum which produce

definite effects on the uterus and other parts of the female body associated with pregnancy and parturition. The existence of female hormones formed in the ovary is now definitely proved. These hormones appear to be formed in the follicles and to be quite distinct from the hormones derived from the corpus luteum (Hisaw). The tunica albuginea of the ovary develops much later than that of the testis but is also of mesenchymal origin.

The genital ducts. — The sperms formed in the seminiferous tubules of the testis are discharged into the mesonephric tubules and thence make their way into the mesonephric duct, which accordingly becomes the male genital duct. The ova, on the other hand, are discharged directly into the cavity of the coelom whence they are received into a new tube, the oviduct, by means of an opening, the ostium tubae (abdominale). The mesonephric duct is often called the Wolffian duct; the oviduct is frequently called the Müllerian duct. Both ducts appear in every embryo (Fig. 135A), but the later histories of the two differ according to the sex.

The Wolffian duct. — In the male (Fig. 135B), the efferent ductules toward the posterior end of the series become occluded, leaving only a few at the anterior end functional. These lose their renal corpuscles and shorten greatly. In the amniotes, where the metanephros acts as the functional kidney, this anterior group becomes the epididymis, while the more posterior, non-functional vestige becomes the paradidymis. The mesonephric duct persists as the deferent duct. At the point where the deferent duct enters the cloaca, there develops a dilation, the seminal vesicle. In the female (Fig. 135C), the anterior portion of the mesonephros persists as the vestigial epoöphoron, and the posterior portion becomes the paroöphoron. Traces of the Wolffian duct sometimes persist, as in mammals, where this structure is known as Gartner's canal.

The Müllerian duct. — This canal arises in the elasmobranchs by the constriction of the pronephric duct into two tubes, of which the ventral becomes the Müllerian duct, while the dorsal tube becomes the Wolffian duct. The opening of the Müllerian duct into the coelom, the ostium tubae abdominale, is a persistent nephrostome. In all other vertebrates, this duct arises independently of and after the formation of the Wolffian duct, a

fact possibly correlated with the delayed functioning of the oviduct as compared with the primary renal function of the Wolffian duct. In these vertebrates the duct arises in the mesoderm lateral to the Wolffian duct and grows both forward and backward until the abdominal and cloacal openings are formed. It is not formed until late in embryogenesis. In the female (Fig. 135C), the posterior ends of the ducts are usually dilated as

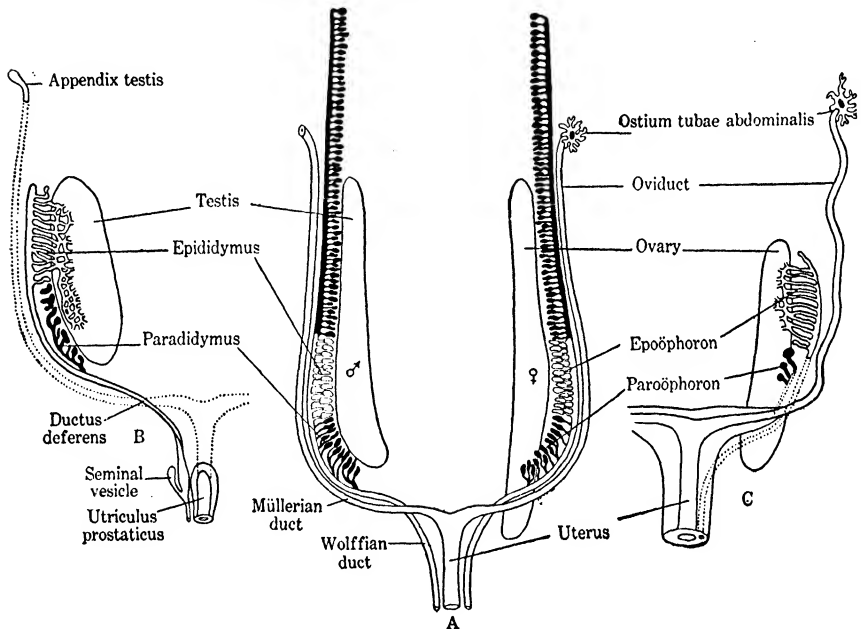


FIG. 135. — Diagrams showing origin and early development of genital ducts. A, early stage showing mesonephros, gonads, (male on left, female on right) and ducts. B, later stage in male, showing in broken lines the structures which degenerate. C, later stage in female. (After Felix.)

storage chambers, and not infrequently fuse to form a uterus. In the male (Fig. 135B), the Müllerian duct degenerates, but vestiges are to be found even in the adult, such as the appendix testis and prostatic utricle of man, which represent the anterior and posterior ends of the female duct, respectively.

Estrous cycle. — Most vertebrates have an annual breeding season. Among the mammals, however, the fact that the young develop for a longer or shorter period (of gestation) in the uterus of the mother is associated with a periodical set of changes in the

activity of the uterus which are known as the estrous cycle. There are three main stages: proestrus, estrus, and anestrus.

During the proestrus the blood vessels of the uterine wall are congested, and in some animals (dog) there is destruction of the uterine wall accompanied by the discharge of blood into the cavity of the uterus.

In estrus the destructive changes of the proestrus are repaired while the cavity itself often contains the secretions of the uterine glands and the materials discharged in the preceding period ("uterine milk"). It is in this period that ovulation usually takes place and the wall of the uterus is in the condition most favorable for the implantation of the blastocyst. The estrus receives its name from the fact that this is the time in which the sexual drive is strongest. If implantation (page 140) and pregnancy do not take place, a condition known as pseudopregnancy occurs in some animals (rat, rabbit, etc.). In the closing stages of the estrus, the wall of the uterus returns to its normal condition, accompanied in some animals (dog) by slight hemorrhages. This period of repair is distinguished (Marshall) as the metestrus.

The estrus is succeeded by the anestrus, a name given to the interval lasting until the next proestrus commences. In many mammals estrus occurs but once during the breeding season, but in others it may take place more frequently. The period between each estrus and the next proestrus is sometimes known as a diestrus in these polyestrous species.

There is a considerable difference of opinion among the authorities as to the exact relation between ovulation and menstruation, a term applied to the periodic hemorrhages characteristic of the female primate. It is assumed that the period of ovulation corresponds to the estrus, but the clinical evidence is not clear as to whether the menstrual discharge is comparable to that of the proestrus or that of the closing stages of the estrus itself.

The external genitalia. — The genital organs so far considered are common to all vertebrates and are sometimes spoken of as the internal genitalia. External genitals are found only in those animals in which fertilization is internal. These organs serve the function of transmitting or receiving the sperm at the time of copulation. Internal fertilization is a phenomenon which has

been observed in all classes of the vertebrates, but it is characteristic of all amniotes.

Although the external genitalia differ in the sexes, they are embryologically homologous. Two types are recognized, duplex and simplex. In the duplex type, characteristic of the sauropsids, sac-like extensions arise on each side of the cloaca, which in the male become the hemipenes or intromittent organ, while in the female they remain vestigial.

In the simplex type, characteristic of mammals, a single median ectodermal prominence arises anterior to the cloacal aperture, to become the phallus (Fig. 136). In the male, the phallus enlarges and encloses the greater part of the urogenital sinus. In this way it becomes the penis, while the enclosed sinus becomes the penile urethra.

In the female mammal, the phallus

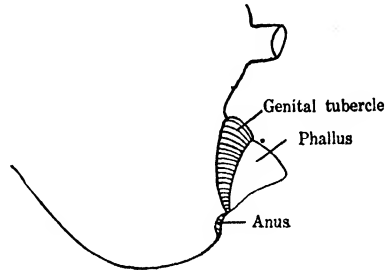


FIG. 136. — Diagram to show the origin of the mammalian external genitalia. (After Felix.)

becomes the vestigial clitoris, while the sides of the urogenital sinus remain open as the labia minora which guard the opening of the urogenital vestibule. At the base of the phallus is a swelling, the genital tubercle, from which labio-scrotal folds arise on either side of the urogenital opening. In the male they fuse to form the scrotum, an external sac into which the testes descend; in the female they remain separate as the labia majora.

TABLE 9
HOMOLOGIES OF THE MAMMALIAN GENITAL SYSTEM

Male	Indifferent	Female
Testis	Gonad	Ovary
Epididymis Paradidymis	Mesonephros	Epoöphoron Paroöphoron
Ductus deferens	Mesonephric (Wolffian) duct	Gartner's canal
Appendix testis Prostatic utricle	Müllerian duct	Uterus Vagina
Penis	Phallus	Clitoris Labia minora
Scrotum	Labio-scrotal swellings	Labia majora

THE FROG (SEE ALSO CHAPTER XI).—The genital ridges arise soon after hatching. Sex can be distinguished at the time when the embryo is about 30 mm. in body length. The anterior portion of each genital ridge degenerates and becomes a fat body.

The Wolffian duct in the male acquires connection with the testis by means of some of the mesonephric tubules (*vasa efferentia*), and serves as the deferent duct as well as the ureter. A seminal vesicle is formed. A rudimentary Müllerian duct appears. In the female the Wolffian duct functions solely as a ureter while the Müllerian duct becomes the oviduct.

No external genitalia are developed.

THE CHICK (SEE ALSO CHAPTER XII).—The genital ridge arises with the mesonephros as the urogenital ridge. Of this the anterior region gives rise to the gonad on the mesial side. Sex is not distinguishable until the seventh day of incubation. In the female, the right ovary develops only partially and finally disappears.

The Wolffian duct becomes the deferent duct, connected with the testis by *vasa efferentia* forming the epididymis. The persisting mesonephric tubules of the posterior region of the mesonephros form a paradidymis. In the female a vestigial epoöphoron and paroöphoron represent these bodies respectively. The Müllerian ducts degenerate in the male without ever acquiring a cloacal exit. In the female the right Müllerian duct disappears while the left becomes the oviduct. The shell gland appears on the twelfth day of incubation, but the cloacal opening is not formed until the hen is six months old.

No external genitalia are formed, although hemipenes are formed in some other birds.

MAN (SEE ALSO CHAPTER XIII).—The genital ridge arises on the mesial side of the mesonephros. Sex is not distinguishable until after the fifth week.

Each Wolffian duct functions as a deferent duct, and both epididymus and paradidymis are formed, as is a seminal vesicle at the distal end. In the female, epoöphoron and paroöphoron are formed, while some portion of the duct itself may persist as Gartner's canal. The Müllerian ducts become the uterine tubes, which unite at their posterior ends to form the uterus and vagina. The latter is partially closed by a semicircular

fold, the hymen, where it enters the urogenital sinus. In the male, vestiges of the anterior end of each Müllerian duct persist as the appendix testis, while the posterior end is represented by the rudimentary prostatic utricle. The dilation of the bladder results in the inclusion of the ureters (metanephric ducts) in its walls. The genital ducts (Wolffian or Müllerian ducts) empty into the urogenital sinus posterior to the bladder, in a region which constricts to form the urethra. About this develop a number of outgrowths which acquire cavities and form the prostate gland in the male, and the para-urethral glands of the female. The external genitalia are of the mammalian type.

D. THE ADRENAL ORGANS

Closely associated with the nephric organs are the mesodermal interrenal glands, which frequently become associated with the suprarenal glands, of ectodermal origin, to form the so-called

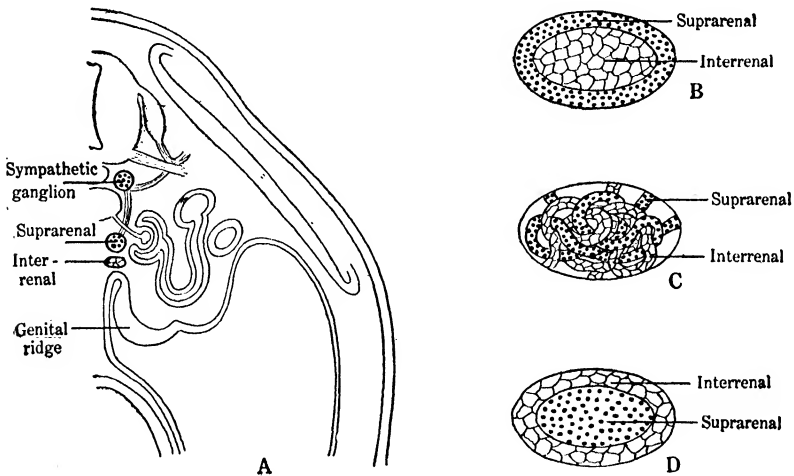


FIG. 137. — Diagrams to show the origin of the suprarenal and interrenal components of the adrenal gland. A, origin as shown in cross section (after Corning). B, condition in amphibia. C, in birds. D, in mammals.

adrenal glands. All are endocrine (or ductless) glands. The suprarenal portion of the adrenal forms the powerful hormone epinephrin (adrenalin); the interrenal portion secretes a hormone known as cortin (Swingle), which is employed in the treatment of Addison's disease.

The interrenals.— These arise as paired thickenings of the splanchnic mesoderm mesial to the nephrocoels. In some of the amphibians there are traces of a segmentation which is soon lost by fusion. There is no direct connection between the interrenal and the mesonephros. These glands may fuse to form an elongate median organ or become associated with the suprarenals.

The suprarenals.— Although these glands are found in the vicinity of the mesonephros, they originate from the sympathetic ganglia (ectodermal) as described in the following chapter. They are separate structures in the fish, but unite with the interrenals in the tetrapods.

The adrenals (Fig. 137).— These compound glands are not found in the fish. In the amphibians the suprarenal portion of the gland is external to the interrenal portion. In the chick they are intermingled. In the amniotes, however, the interrenal substance (cortex) surrounds the suprarenal (medulla).

E. THE VASCULAR SYSTEM

The vascular system is mesenchymatous in origin. It consists of separate cells, the blood corpuscles, floating in a fluid matrix,

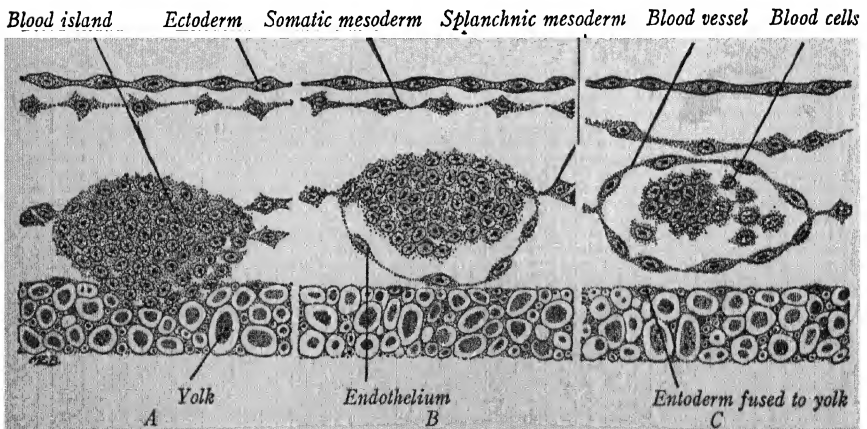


FIG. 138. — Diagrams showing three stages in the development of capillary from blood island based on transverse sections of the area vasculosa in a seven somite chick. (From Arey.)

the blood plasma, in a closed system of interconnected tubes, the blood vessels. Some vessels become lined externally with muscle fibers, and in one locality this muscular development gives rise

to a pulsating heart by means of which the blood is kept in circulation.

Origin of the blood-vascular system. — The first indications of the vascular system are found in the splanchnopleure as blood islands (Fig. 138). In the telolecithal vertebrates this is always in the extra-embryonic splanchnopleure. These blood islands originate as local aggregates of mesenchyme. Later, the inner cells separate as corpuscles, while the outer ones form the endothelial lining of a vesicle. These vesicles anastomose with each other to form the extra-embryonic vitelline circulation.

The blood corpuscles. — The first corpuscles formed are the inner cells of the blood islands. Later corpuscles are budded off

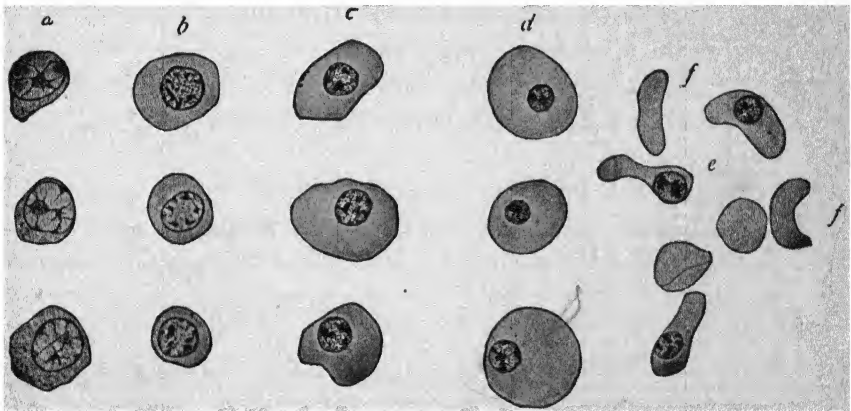


FIG. 139. — Stages in the development of human red blood corpuscles. A, hemoblasts. B, megaloblasts (anamniote type). C, D, normoblasts (sauropsid type). E, normoblasts in process of becoming F, erythrocytes. (From Arey after Prentiss.)

from the walls of the capillaries into their cavities. Mesenchymal cells in regions where the capillary network is forming may develop into blood corpuscles and enter the blood stream. These first corpuscles are the hemoblasts (Fig. 139).

Hemoblasts become differentiated into the different types of blood corpuscles in the following blood-forming centers: (1) the yolk sac; (2) the embryonic capillaries; (3) the liver, the spleen, and the lymph glands; (4) the bone marrow. In the adult the lymph glands give rise to lymphocytes, and the bone marrow to all types of corpuscles.

The erythrocytes, or red corpuscles, are distinguished by the presence of hemoglobin which gives them their color. In the

anamniotes the erythrocytes have a large vesicular nucleus with granular chromatin and a distinct cell membrane. In the sauropsida, the erythrocytes have a small compact nucleus. The mammalian erythrocyte is distinguished by the absence of the nucleus in the adult. In the development of mammals there is a succession of erythrocytes: first the anamniote type; then the sauropsid type; and finally the mammalian erythrocyte, which is produced by the extrusion of the nuclei from the blood cells of the sauropsid type (Fig. 139).

The leucocytes, or white corpuscles, are of many types, for a discussion of which the reader is referred to the textbooks on histology. The preponderance of evidence indicates that these, like the erythrocytes, are derived from the hemoblasts.

Origin of the intra-embryonic vessels. — The first embryonic blood vessels (Fig. 140) are the vitelline veins which appear at the ventro-lateral margins of the fore-gut. These vessels unite in the region of the anterior intestinal portal to form the heart, then separate as the ventral aortae, which bend up around the pharynx in the mandibular arch as the first aortic arches, and continue backward as the dorsal aortae. These fuse at a very early stage as the dorsal aorta, from which branches are sent to each myotome and to the vitelline circulation. The posterior ends of the vitelline veins fuse in small-yolked forms, such as the frog, to form a subintestinal vein which continues back to the tail. In large-yolked forms like the chick, the vitelline veins are widely separated and brought into connection only by the sinus terminalis which makes a circuit of the area vasculosa. The vitelline veins are the ventral venous channels of the splanchnic circulation. A dorsal set of vessels soon originates independently to form the somatic venous circulation. The first of these to appear are the anterior cardinal (precardinal) veins of the head. A similar pair, the posterior cardinal (postcardinal) veins, arise in connection with the nephric region. These, however, do not discharge their contents directly into the heart but into the anterior cardinals. The portions of the original anterior cardinals proximal to this juncture with the posterior cardinals are now called the common cardinal veins.

The heart. — Although the heart is primitively a paired organ, we have seen that the two primordia are soon fused into a single

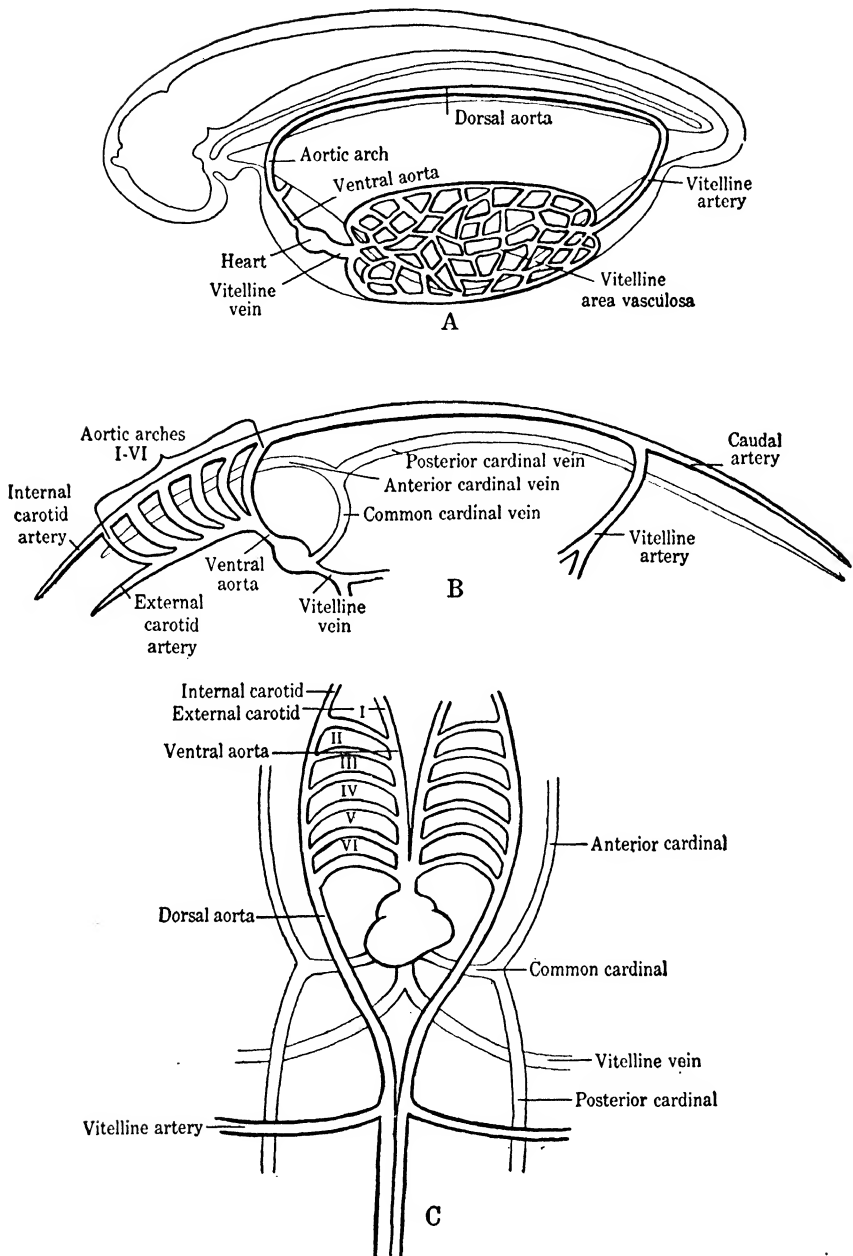


FIG. 140. — Diagrams to show fundamental plan of embryonic circulation. A, early stage in side view. B, later stage in side view. C, same from above, aortic roots pulled apart.

median tube connected with the ventral aortae in front, and the vitelline veins (and later the common cardinals) behind. Around the endocardial lining there develops a coat of muscle fiber which later becomes striated to form the myocardium. Outside this is a lining of splanchnic mesoderm which forms the epicardium, continuous with the lining of a part of the coelom surrounding the heart, which will later be cut off by the septum transversum to form the pericardium. In this the heart is suspended by a dorsal and a ventral mesentery known respectively as the dorsal and ventral mesocardia.

The later history of the heart is one of growth and subdivision into special chambers. Because the local growth of the heart is limited by the anterior and posterior walls of the pericardium

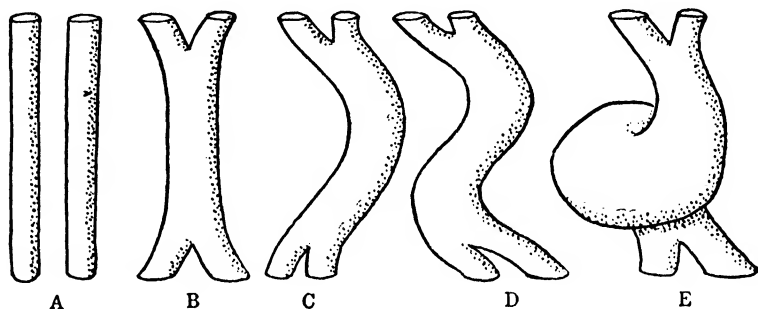


FIG. 141. — Diagrams to show early stages in development of vertebrate heart. A, paired heart tubes. B, same fused. C, primary flexure. D, later "S" stage. E, after antero-dorsal displacement of atrium.

and by the mesocardia in which it is suspended, any extension in length must be accompanied by coiling. The primary flexure of the heart is toward the right, thus changing the shape of the organ from a straight tube to a C-shaped one. Further growth results in the twisting of the heart into the shape of an S. Still later, the original posterior loop of the S is pushed forward and dorsad so that it comes to lie above the morphologically anterior end (Fig. 141).

The original chambers of the heart are produced by local dilations, of which the most posterior is the sinus venosus; next to this is the atrium; in front of this, the ventricle; and finally, the bulbus arteriosus. The sinus is the chamber into which the primitive veins enter; the atrium is a thin-walled distensible

chamber; the ventricle is a thick-walled, muscular, pulsating pump; and the bulbus is the chamber from which the blood enters the primitive arteries.

These chambers undergo different changes in the various types of vertebrates. Of these, the most important is a progressive differentiation, completed in the mammals and birds, of the atrium and ventricle into separate right and left halves, of which the right side receives venous blood from all parts of the body and transmits it to the lungs for respiratory exchange. From the lungs the blood is returned to the left side of the heart and thence conveyed to all parts of the body.

The arteries (Fig. 142). — The ventral aortae fuse into a single median tube sending branches into each of the visceral arches.

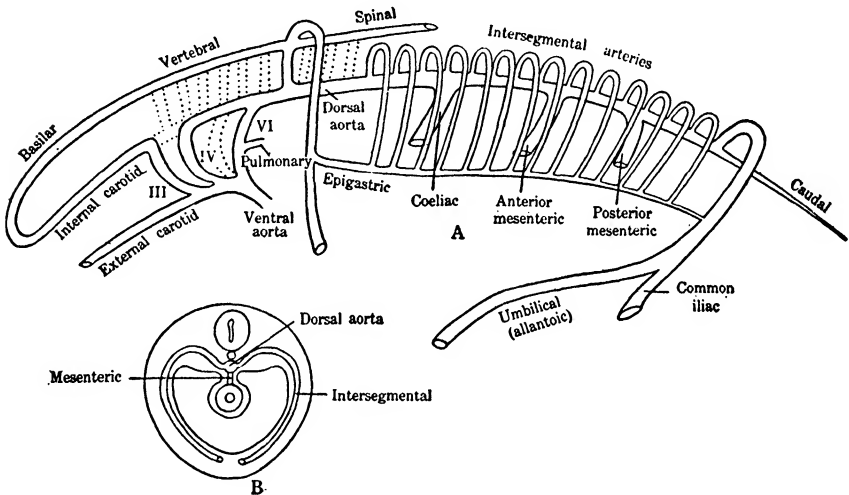


FIG. 142. — Diagrams to show principal arteries; A, in side view, B, cross section through mesenteric.

These branches, which unite with the dorsal aortae, are usually six in number and are known as the aortic arches. Anterior to these the ventral aortae continue forward as the external carotid arteries. Similar forward extensions of the dorsal aortae are known as the internal carotid arteries. In the region of the aortic arches the dorsal arteries remain separate as the aortic roots (*radices aortae*). Behind them, as has been mentioned, the paired vessels fuse as the median dorsal aorta.

The aortic arches. — In larvae breathing by means of external gills, a loop from each aortic arch grows out into the gill developing on the visceral arch with which it is associated. These loops are short-circuited when the external gills disappear.

In forms with internal gills, each aortic arch breaks up into capillaries in the demibranch and becomes divided into a ventral afferent branchial artery and a dorsal efferent branchial artery.

In vertebrates with a pulmonary respiration, aortic arches I and II, in the mandibular and hyoid arches, respectively, disappear. Arch III, in the first branchial arch, persists as the connection between the internal and external carotid arteries,

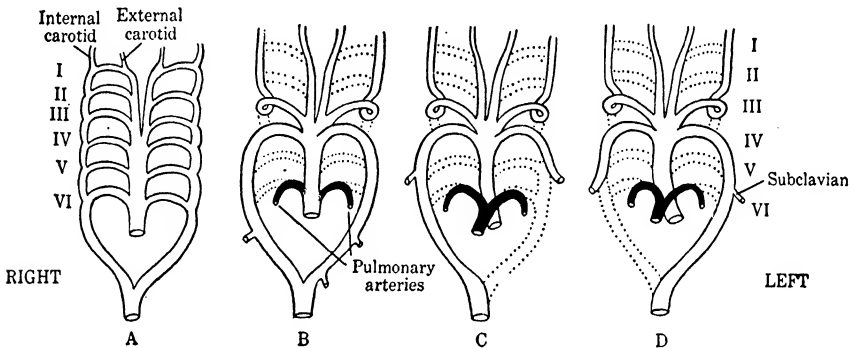


FIG. 143. — Diagrams of aortic arches. A, hypothetical primitive type. B, in frog. C, in chick. D, in man. (After Kingsley.)

while the dorsal aorta between arches III and IV disappears. Arch IV becomes the systemic arch connecting the dorsal and ventral aortae (Fig. 143B). In birds (Fig. 143C), the arch on the left side disappears; in mammals (Fig. 143D), that on the right degenerates. Arch V is greatly reduced and frequently disappears or has at most a vestigial and transitory existence. From arch VI there grow back to the lungs the pulmonary arteries. The portion of the sixth arch distal to the pulmonary arteries is reduced in caliber and is known as the ductus arteriosus. It becomes occluded and degenerates in all the amniotes except some few reptiles.

Intersegmental arteries. — From the dorsal aortae are given off small branches between the myotomes (Fig. 142B). Some of these intersegmental arteries persist as the intervertebral arteries. The more anterior ones become united on either side by

a dorsal longitudinal vertebral artery. These vertebrals subsequently fuse to form an anterior basilar artery which divides behind the pituitary, the two halves uniting with the internal carotid on either side. The posterior halves of the vertebral arteries fuse to form the spinal artery which runs back beneath the spinal cord. In the region where the anterior limb buds are developing, intersegmental arteries grow out, to give rise to the subclavian arteries. Similarly, in the region of the pelvic limb buds, intersegmental arteries give rise to the iliac arteries. In the amniota, the allantoic arteries grow out from the iliac arteries into the walls of the allantois. These become so important that for some time it appears as though the iliac arteries were derived from the allantois instead of the reverse. These allantoic arteries, which degenerate at the time of birth, are known as the umbilical arteries in mammals as they traverse the umbilical cord and supply the placenta.

Other important intersegmental arteries become the renal arteries of the kidneys and the genital arteries of the gonads.

Mesenteric arteries. — From the dorsal aorta, a number of ventral branches, originally paired, but soon fused to become median vessels, pass down the dorsal mesentery. They unite with the capillaries of the yolk sac which they supply with blood. Later, some of them develop branches over the alimentary canal which persist after the loss of the yolk sac as the coeliac and mesenteric arteries.

The veins. — There are two primitive venous systems: the somatic system, comprising the cardinal veins; and the splanchnic, including the vitelline (omphalomesenteric) and, in amniotes, the allantoic (umbilical) veins. The cardinal veins are replaced by caval veins; the vitelline veins become transformed into a hepatic-portal system. The allantoics disappear at hatching (or birth). Finally, there are the pulmonary veins. In general, the history of these transformations may be summed up in the statement that the primitive independent venous systems become transformed into a system wherein an accompanying vein is developed for every artery.

The vitelline veins (Fig. 144). — These vessels, and their continuation, the subintestinal vein (in small-yolked forms), are the first vessels formed in the embryo. In the amniotes, two veins

grow out from these into the wall of the allantois to become the allantoic veins of the sauropsida (umbilicals of mammals). In man, however, the umbilical veins actually appear before the vitelline veins.

It has been noted previously that the vitelline veins pass around the liver on their way to the heart. As the liver enlarges, it surrounds the vitelline veins, and these become broken up in the liver tissue to form a great capillary network. In the amniota, the allantoic (umbilical) veins are similarly absorbed. The proximal portions of the vitelline veins, from the liver to the sinus venosus, are now known as the hepatic veins; the distal portions

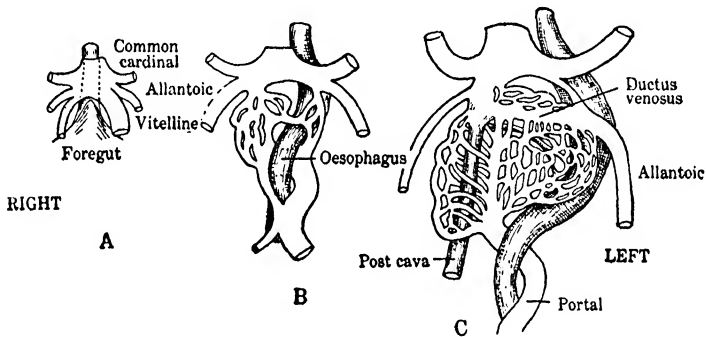


FIG. 144. — Diagrams to show three stages in the development of the hepatic-portal venous system, based on conditions in man. (After Hochstetter.)

are called the portal veins. Of the umbilical veins, the right degenerates; the left for a time maintains a direct connection through the liver to the hepatic veins, known as the ductus venosus. This connection disappears at the time of birth. After the disappearance of the yolk, the portal vein and its tributaries, of which the most important is the mesenteric vein, carry blood from the digestive canal to the liver.

The anterior cardinal veins. — The original plan of the cardinal system is that of an H in which the upper limbs represent the anterior cardinals; the cross-bar the common cardinals, with the heart in the middle of the cross-bar; and the lower limbs represent the posterior cardinals (Fig. 145). The anterior cardinals arise as a drainage system for the blood passing into the head from the carotid arteries.

The anterior cardinals are often called the internal jugular

veins. From these, parallel veins, known as the external jugular veins, branch off in the ventral region of the head. Veins from the vertebral region (vertebral veins) and from the pectoral appendages (subclavian veins) soon develop. In most vertebrates the common cardinals and the proximal portion of the anterior cardinals, i.e., up to the point where these tributary veins diverge, persist as the precaval veins. In some mammals, a cross-connection is formed between the anterior cardinals, after which the portion of the left anterior cardinal, proximal to the anasto-

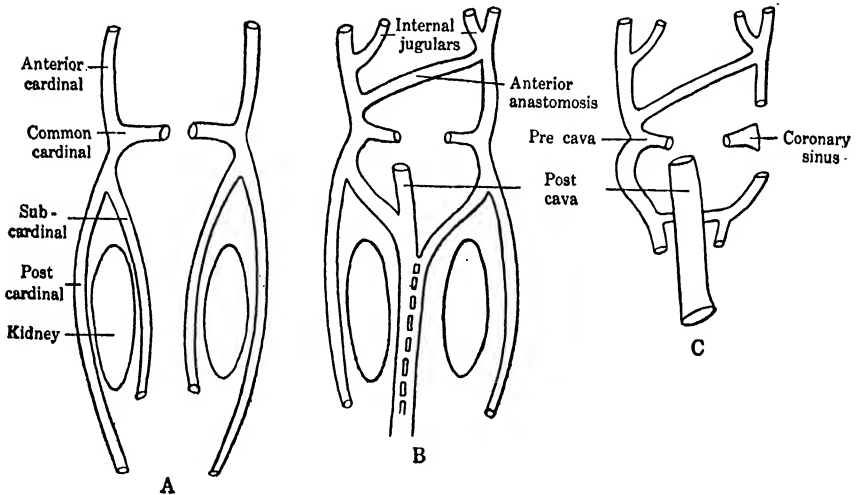


FIG. 145. — Diagrams to show three stages in the development of the caval venous system. Generalized (supra-cardinals omitted).

mosis, and the left common cardinal become the coronary vein draining the wall of the heart. The corresponding vessels on the right side persist as the precaval (anterior caval) vein.

The posterior cardinals. — Each posterior cardinal lies dorsal to the mesonephros which it drains. Beneath each mesonephros is developed a subcardinal vein. In the anamniotes these veins arise as tributaries of the posterior cardinals, returning blood from the tail where they are united to form the caudal vein. Later, they lose direct connection with the parent vessels and return blood from the tail region to the mesonephros as the renal-portal veins. The posterior portions of the subcardinals fuse as the interrenal vein, which acquires a secondary connection with the

hepatic vein, and persists as the postcaval vein. In the amniotes the postcaval vein is a complex which arises partly from the hepatic veins, partly from appropriated portions of the posterior cardinals and subcardinals, and partly from the supra-cardinals, a pair of vessels dorso-mesial to the posterior cardinals. It eventually replaces the posterior cardinals, so that the only blood vessels entering the right side of the heart are (1) the pre-caval vein returning blood from the head, pectoral region, and appendages; and (2) the postcaval vein returning blood from the trunk and pelvic appendages as well as all blood from the digestive canal conveyed by way of the hepatic-portal system.

The pulmonary veins. — These enter the left atrium and are new vessels which grow backward from the heart to the developing lungs.

The lymphatic system. — This system serves to return to the veins the blood plasma which has escaped from the capillaries (Fig. 146). It contains white blood corpuscles of the ameboid type (lymphocytes) which have the power of making their way through the capillary walls. The lymphatics apparently originate as intercellular spaces in mesenchyme which later become confluent and acquire a limiting endothelium. Like the blood vessels, the lymphatic capillaries anastomose and form larger vessels which drain into the veins. The walls of these central vessels are often muscular, and localized areas known as lymph hearts are found. So, too, localized distensible sacs, the lymph sacs, are not unknown. Some of these become lymph glands. The spleen, already alluded to in the section on mesenteries (page 198), is a hemolymph gland in which both lymphocytes and erythrocytes are proliferated.

THE FROG (SEE ALSO CHAPTER XI). — In the frog (Fig. 147), the primordia of the vitelline veins first appear and grow together as a loose aggregate of cells in front of the liver. Around this the coelom grows in from right and left to form the pericardium. Meantime the primordium of the heart endocardium develops from the loose aggregate of cells referred to above. The inner wall of the coelom (splanchnic mesoderm) becomes the myocardium. The atrium is divided by an interatrial septum into two auricles, right and left. The ventricle remains a single chamber.

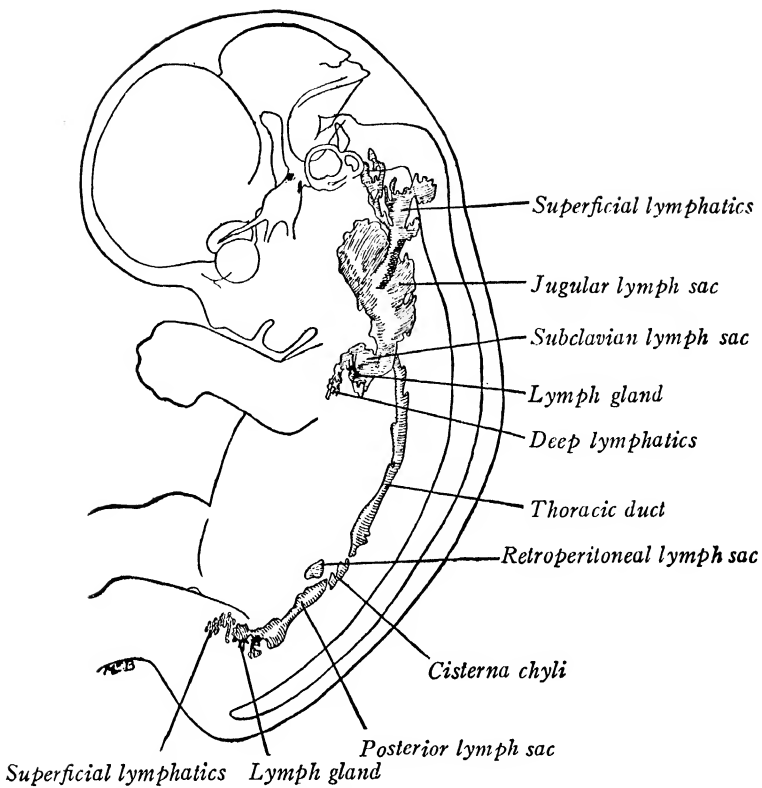


FIG. 146. — Reconstruction of primitive lymphatic vessels in human fetus of two months. (From Arey after Sabin.)

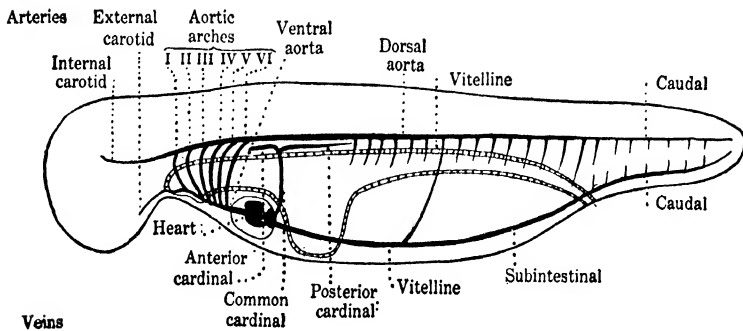


FIG. 147. — Diagram of embryonic vascular system of early tadpole. (After Kingsley.)

Aortic loops develop in the external gills, corresponding to aortic arches III, IV, and V. After the appearance of the internal gills, the ventral limb of the loop becomes the afferent branchial artery, while the dorsal limb becomes the efferent branchial artery. A similar differentiation takes place in arch VI. With the loss of branchial respiration, arch III becomes the proximal portion of the carotid arteries, arch IV the systemic arch which persists on both sides, and arch V disappears, while from arch VI arise vessels which carry blood to both the lungs (pulmonary arteries, Fig. 143B) and skin (cutaneous arteries).

The vitelline veins anterior to the liver fuse to become the hepatic vein: posterior to the liver, the right vitelline vein disappears, the left becomes the hepatic-portal vein. The anterior cardinal veins become the internal jugular veins; the common cardinals become the precaval veins. The posterior cardinal veins fuse between the mesonephroi, and a new vein grows back from the hepatic vein to the right posterior cardinal, to form the postcaval vein. The posterior cardinals, anterior to their junction with the postcaval, degenerate. Posterior to this junction they persist as the renal-portal veins carrying blood from the iliac veins to the kidneys.

THE CHICK (SEE ALSO CHAPTER XII). — In the chick (Fig. 148), the endocardium of the heart arises as the forward extension of the vitelline veins, which soon fuse as the pericardial primordia are brought together beneath the head. The myocardium is formed as in the frog. The right and left halves of the heart are completely separated by three septa: the septum aortico-pulmonale, which divides the bulbus into a chamber on the right leading to the pulmonary arteries and one to the left leading to the dorsal aorta; the interventricular septum, which divides the ventricle; and the interatrial septum, which divides the atrium into two auricles. This separation is completed at the end of the first week of incubation. The sinus venosus is incorporated in the right auricle.

Six aortic arches are formed: I and II disappear on the third and fourth days of incubation; III forms the proximal portion of the internal carotid artery; IV disappears on the left side but persists as the systemic arch on the right; V disappears; the pulmonary arteries arise from VI, but the distal portion of the

right arch remains as the ductus arteriosus until the chick hatches (Fig. 143C).

The vitelline veins unite behind the sinus venosus to form the meatus venosus which later becomes the hepatic vein. The mesenteric vein becomes the portal vein, and the vitelline veins disappear at hatching. The allantoic veins grow backward from the common cardinals to join the capillaries of the allantois; the right allantoic degenerates on the fourth day, and the left acquires a new connection with the meatus venosus, by way of the

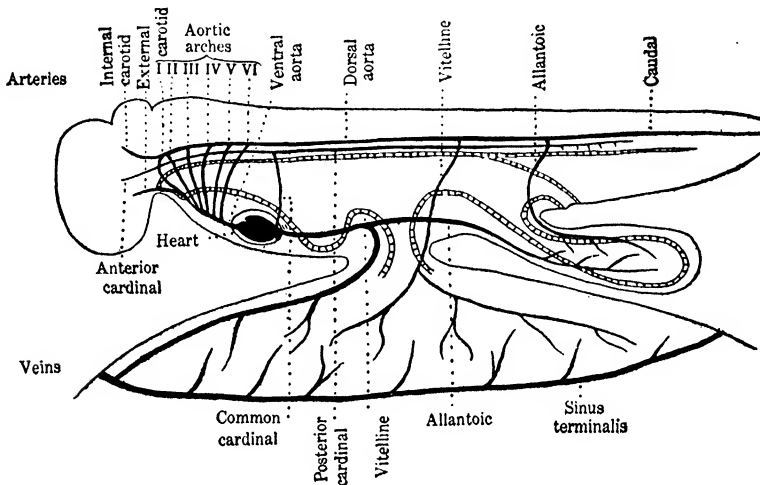


FIG. 148. — Diagram of embryonic vascular system of chick. (After Kingsley.)

left hepatic vein. The allantoic vein degenerates at hatching. Two precaval veins are formed from the proximal portions of the anterior cardinals and common cardinals. The posterior caval vein arises from (1) a branch of the meatus venosus which grows back to meet the right subcardinal vein, (2) the fused subcardinals which carry blood from the mesonephros, and (3) the renal veins which develop in connection with the metanephros. The anterior ends of the posterior cardinals disappear, while the posterior ends supply the mesonephros and, after its degeneration, the common iliac veins, which pass directly to the post-caval vein.

MAN (SEE ALSO CHAPTER XIII).— The heart arises in man (Fig. 149) much as in the chick; but the subsequent partition-

ing of this organ into right and left halves is more complicated, for two atrial septa are formed. The ventricle is separated by an interventricular septum, and the bulbus is divided by two septa which unite to form the septum aortico-pulmonale. The sinus venosus is incorporated in the right atrium.

The aortic arches are formed and have the same history as those of the chick, with the exception that it is the left fourth aortic arch which becomes the systemic arch (Fig. 143D).

The anterior portion of the right vitelline vein becomes the hepatic vein; the hepatic-portal arises from the posterior portion of the vitelline veins anterior to their junction with the mesenteric

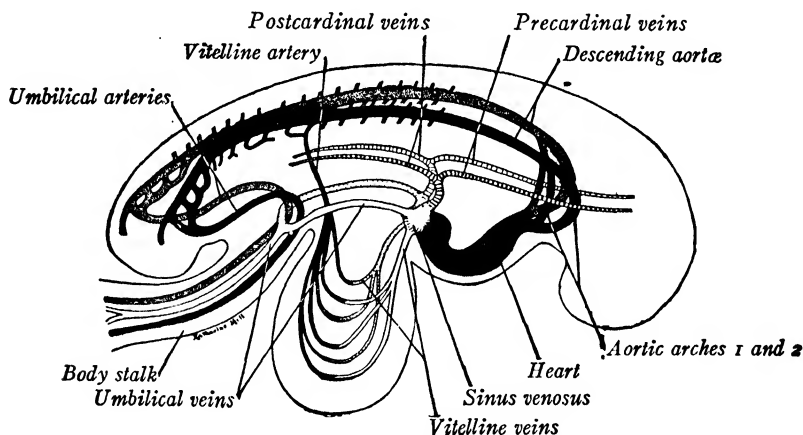


Fig. 149. — Diagram of embryonic vascular system in man: (From Arey after Felix.)

vein. The anterior cardinals are united by an anastomosis (left innominate vein), and the left common cardinal disappears with the exception of the coronary vein. The right common cardinal, together with that portion of the anterior cardinal as far as the branching of the left innominate, becomes the precaval vein. The postcaval vein is a complex formed from (1) a branch of the hepatic vein, (2) the anterior portion of the fused sub-cardinals, (3) part of the fused supracardinals, and (4) the fused posterior portion of the posterior cardinals. The anterior portions of the posterior cardinals separate from these veins, unite by means of an anastomosis, and drain into the right precaval vein. They are then known as the azygos (right) and hemiazygos (left) veins. Of the umbilical veins, the left only persists, with a

direct connection through the liver by means of the ductus venosus. At birth this duct closes and the umbilical vein disappears.

F. THE SKELETON

The skeleton of vertebrates consists of a system of supporting and protecting elements developed from mesenchyme. These elements pass through several conditions in later development. The primordia of the skeletal elements are preformed in connective tissue. These become transformed into cartilage, a process known as chondrification, through the activities of specialized cells, the chondrioblasts. Cartilage in turn is transformed into bone, through the action of osteoblasts, the process being known as ossification. Bones that pass through these three stages are known as cartilage bones. In the formation of some bones, the cartilaginous stage is omitted; these are known as membrane bones. Both cartilage and bone are typically surrounded by a membrane of mesenchyme which is called the perichondrium or periosteum, as the case may be. The separate elements of the skeleton are connected with each other by ligaments, by cartilage, or in a bony union.

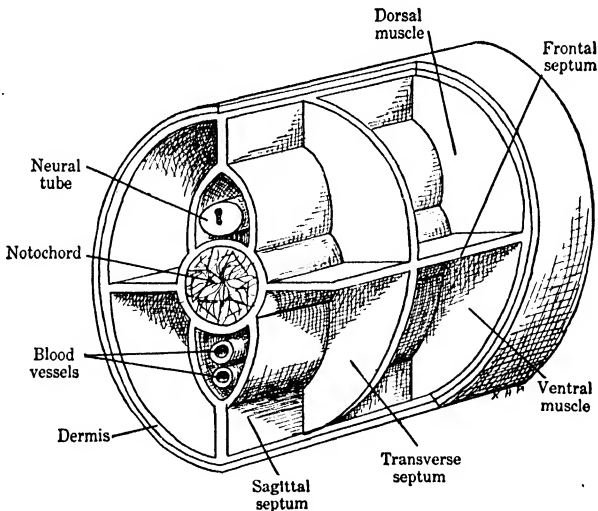


FIG. 150. — Diagram to show the skeleton-forming regions as seen in the tail region of a vertebrate. (After Kingsley.)

Skeletogenous regions. — The principal regions where skeleton may be formed in the vertebrate body (Fig. 150) are (1) the

dermis of the skin, (2) the median sagittal planes between the myotomes on the dorsal and ventral sides of the body, (3) the right and left frontal planes between the dorsal and ventral muscle masses, (4) the transverse planes between the myotomes, (5) around the notochord, neural tube, and axial blood vessels, (6) in the visceral arches, and (7) in the paired appendages. Skeletal elements formed in (1) are called the dermal skeleton; those formed in (2) to (5), the axial skeleton; those formed in (6), the visceral skeleton; and those formed in (7), the appendicular skeleton. The skull contains elements from all but the appendicular skeleton.

The dermal skeleton. — Among living vertebrates the most primitive example of derm bones are the placoid scales (Fig. 151)

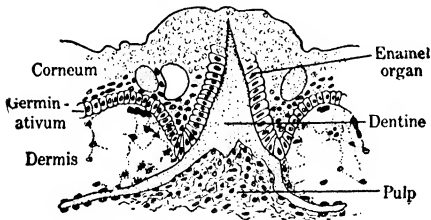


FIG. 151. — Section of developing placoid scale (*Squalus acanthias*) to show origin of primitive dermal bone. Compare Fig. 119. (After Kingsley.)

of the cartilage fish which are formed in exactly the same way as teeth (Chapter VIII). In the dermal skeleton two types of bones are distinguished. The investing bones (dermal plates) serve to envelop regions of the head and trunk. The substituting bones become so closely allied with the cartilaginous bones as

to become fused with them or even to replace them in ontogeny. Many of the cranial bones are of this type. They may be distinguished by the fact that they do not pass through a cartilaginous stage in development.

The axial skeleton. — The primitive axial skeleton is the notochord, whose origin has been discussed in Chapter V. Around this a connective tissue sheath is formed by mesenchymal cells. The mesenchyme

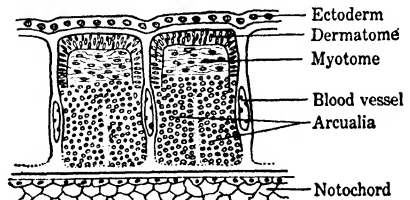


FIG. 152. — Section through sclerotome of lizard (*Sceloporus*) to show arcualia. (After Kingsley.)

from each sclerotome now forms four little blocks, the arcualia (Fig. 152), two dorsal to the notochord and two ventral, from which the arches and centra of the vertebrae are formed, as well

as the primordia of the ribs. The posterior arcualia of each somite unite with the anterior arcualia of the succeeding myotome to form the definitive vertebra, which thus comes to lie at the point of separation between two myotomes. Eight elements are thus concerned with a single vertebra: right and left dorsal arcualia from the anterior half sclerotome, and from the posterior half sclerotome, and the corresponding ventral elements.

The vertebrae. — In the prevertebral masses so formed appear centers of chondrification, one on each side of the spinal cord and one or more below the cord. These form, respectively, the neural arch and the centrum of the vertebrae (Fig. 153). In the tail region, two centers of chondrification arise below the centrum,

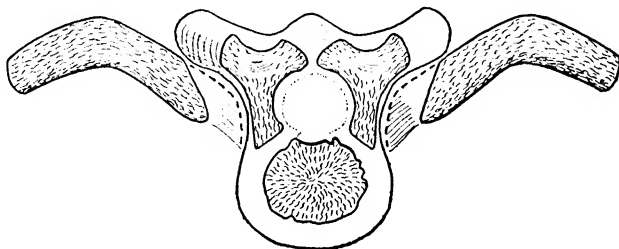


FIG. 153. — Section to show ossification centers in human vertebra and ribs. (After Kollman.)

enclosing the caudal prolongation of the dorsal aorta, and form a hemal arch. With the chondrification of the vertebrae the notochord disappears in all but the most primitive vertebrates, persisting only between the vertebrae as nuclei pulposi of the intervertebral discs. Finally the vertebrae become ossified, and the spines, zygapophyses, and other differentiations are developed.

The ribs. — Except in the caudal region, lateral processes arise from the vertebral primordia and grow out into the myosepta. They later become cartilaginous, and finally true bone. These are the ribs, of which there are two types, dorsal and ventral, distinguished according to the part of the vertebra from which they originate.

The sternum. — The sternum, or breast bone, arises in the amphibians from the coalescence of two longitudinal bars of cartilage, which later articulate with the coracoids of the pectoral girdle, but do not come in contact with the ribs. In the amniota,

the sternum arises from the fusion of the ventral ends of the anterior rib rudiments. In this way there arise two longitudinal bars, from which the unpaired sternum is formed by fusion along the mesial line (Fig. 154).

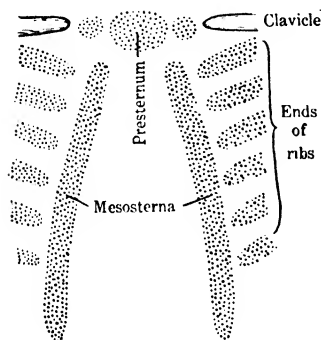


FIG. 154. — Diagram to show origin of mammalian sternum. (After Kingsley.)

The skull. — The skull is a complex of skeletal elements, arising from the chondrocranium, or primitive cranium of cartilage bones, which is derived in part from the protective covering of the brain and sense organs (neurocranium), and in part from the supporting elements of the visceral arches (splanchnocranium). This is supplemented by numerous investing and substituting bones from the original

dermal skeleton (dermocranium).

Neurocranium. — The neurocranium arises from the head mesenchyme which, as has been said, cannot be traced to any definite somites. In this mass, which completely invests the brain and sense organs, definite centers of chondrification appear. These masses unite to form the chondrocranium of the cartilage fish (Fig. 155). If the notochord be used as a point of orientation,

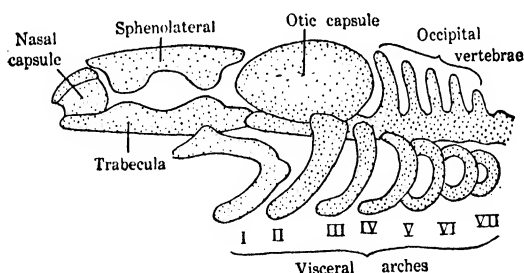


FIG. 155. — Diagram showing components of chondrocranium (*Squalus acanthias*). (After Kingsley.)

on either side of it is found a parachordal bar. In front of each of these is a separate rod; these are the trabeculae. Between the two parachordals and around the notochord, the basilar plate arises as the support of the epichordal brain. The trabeculae also fuse in front, to form the ethmoid plate which supports

the prechordal brain, but remain separate at their posterior ends to form an opening through which the pituitary projects downward. In front of the ethmoid plate the trabeculae grow forward as the cornua. Dorsal to each trabecula, another longitudinal bar, the sphenolateral, arises. Between these two bars the cranial nerves make their way to the exterior.

Around each of the major sense organs a cartilaginous capsule develops. The olfactory capsules unite with the cornua, ethmoid, and sphenolaterals. The optic capsule rarely develops fully, usually persisting in the connective tissue stage as the sclera of the eyeball. The otic capsule, however, becomes completely chondrified and unites with the parachordals and the latero-sphenoids. Between the two otic capsules and sphenolaterals arises a dorsal plate which forms a roof for the brain. In the amniotes, one or more neck vertebrae are consolidated with the occipital region.

The splanchnocranium. — The digestive canal in the head region consists of the mouth, oral cavity, and pharynx, the walls of the pharynx being penetrated by the visceral clefts. As there is no coelom in this region, the lateral mesoderm is not divided but gives rise to mesenchyme which foreshadows the cartilaginous bars supporting the wall of this part of the body. These visceral arches are the mandibular, hyoid, and four (or more) branchial arches. The mandibular arch divides into dorsal and ventral portions, of which the dorsal portion becomes the pterygoquadrate cartilage (upper jaw of cartilage fish) while the ventral portion becomes the meckelian cartilage (lower jaw). The hyoid arch divides into a dorsal hyomandibular cartilage, and a ventral hyoid cartilage which is usually divided into several centers of chondrification. The hyomandibular acts as a suspensory element for the jaws in the fish. It is homologized with a bone of the middle ear, the columella, in amphibians, and the stapes of mammals (see page 269). The hyoid gives rise to the support of the tongue. The branchial arches are usually divided into four parts and act as gill supports in the anamniota and disappear or become laryngeal cartilages in amniota.

Ossification of the chondrocranium. — The limits of this text will not permit of an enumeration of all the bones formed from the chondrocranium (Figs. 156, 157, 158). They may be grouped,

however, as follows: (1) the occipitals, formed from the occipital vertebrae; (2) the sphenoids, arising from the parachordals, basilar plate, trabeculae, and latero-sphenoids; (3) the ethmoids,

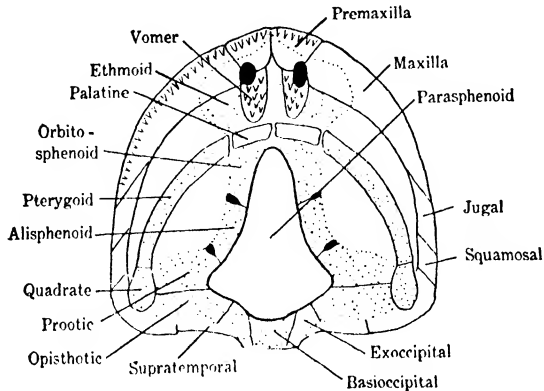


FIG. 156. — Diagram showing components of vertebrate skull, generalized. Ventral view. Chondrocranium stippled, dermal elements in outline. (After Kingsley.)

from the ethmoid plate and nasal capsule; (4) the otics, from the otic capsule. The pterygoquadrate bar gives rise to the pterygoid bones and the quadrate (which in mammals becomes the incus of the middle ear). The meckelian cartilage gives rise to the

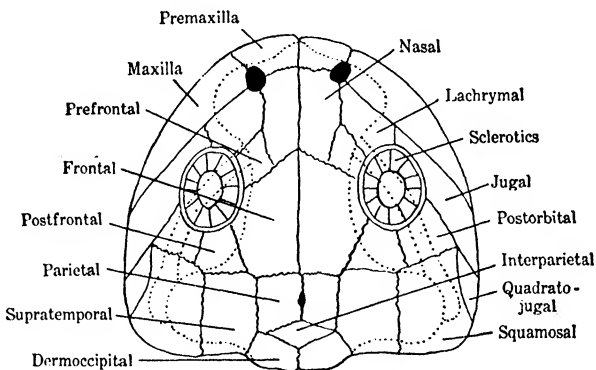


FIG. 157. — Dorsal view of skull diagrammed in Fig. 156.

articular bone at its distal extremity. This becomes the malleus, another ear-bone, of the mammals. The remainder of the meckelian persists as cartilage. In the hyoids and the branchials, bones are formed which retain the names of their cartilaginous predecessors.

The dermocranium (Figs. 156, 157, 158). — The derm bones which invest and, to some extent, supplant the elements of the chondrocranium are too numerous to be more than mentioned here. The dorsal derm bones are, from front to rear, the nasals, frontals, and parietals, together with a number of smaller bones which appear in variable quantity in the different classes. The principal lateral elements, from front to rear, are the premaxillae,

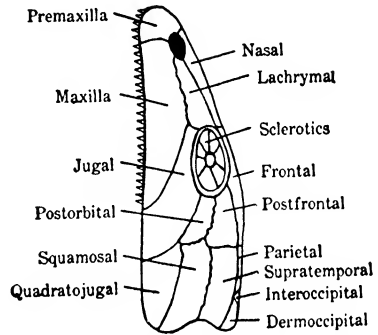


FIG. 158. — Lateral view of skull diagrammed in Figs. 156, 157.

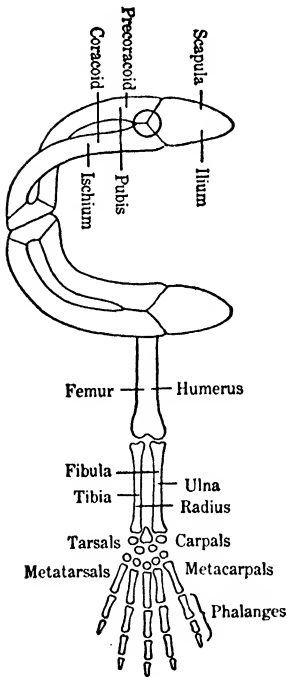


FIG. 159. — Diagram of appendicular skeleton, tetrapod type, showing homologies of pectoral elements above and to left; pelvic elements below and to right. (After Kingsley.)

maxillae, jugals, quadratojugals, and squamosals. The floor of the chondrocranium is invested by the parasphenoids, palatines, and vomer. The lower jaw is invested by a series of bones of which the most important is the dentary.

The appendicular skeleton. — The simplest forms of appendages, the unpaired and paired fins of fish, contain a skeleton consisting of parallel cartilaginous rods, which are divided into proximal portions, basalia, embedded in the body, the distal portions, radialia, extending into the free appendages. The paired appendages of fish are paddle-like fins; in tetrapods they are jointed legs. In both, the skeleton is divided into a basal girdle and a free appendicular skeleton (Fig. 159).

The girdles. — The girdles are in the form of inverted arches, of which the pectoral girdle is united to the axial skeleton in fish and free in the tetrapods, while the pelvic girdle, usually free in fish, is united to the axial skeleton in the tetrapods. Each arch

typically consists of three portions. The dorsal one in the pectoral girdle is the scapula; in the pelvic girdle it is called the ilium. The two ventral elements of the pectoral girdle are the precoracoid (anterior) and the coracoid (posterior), while the corresponding elements of the pelvic girdle are the pubis and ischium. In the shoulder region, the clavicle, a derm bone, becomes associated with the pectoral girdle.

The free appendages. — The pectoral and pelvic appendages are very similar. Each has three segments: proximal, intermediate, and distal. The proximal segment of the pectoral appendage contains one bone, the humerus, while the corresponding bone of the pelvic limb is called the femur. The intermediate portion of the pectoral limb possesses two bones, the radius and ulna; while the corresponding bones of the pelvic appendage are the tibia and fibula. The distal segment is divided into three regions of which the proximal portion contains nine or ten bones, the carpalia of the pectoral appendage, tarsalia of the pelvic. The intermediate portion contains five metacarpalia or metatarsalia, respectively. The distal portion contains the free phalanges of the fingers or toes.

TABLE 10
HOMOLOGIES OF APPENDICULAR SKELETON

Pectoral	General	Pelvic
Scapula Procoracoid Coracoid	Girdle	Ilium Pubis Ischium
Humerus Radius Ulna Carpalia Metacarpalia Phalanges (I-V)	Free appendage	Femur Tibia Fibula Tarsalia Metatarsalia Phalanges (I-V)

Origin of the appendicular skeleton. — All the bones of the appendicular skeleton, with the exception of the clavicle, are formed from a mesenchymal blastema in the limb buds by the appearance of centers of chondrification. The origin of this

mesenchyme is probably from the somites, but the details of the process are still imperfectly understood.

THE FROG.¹ — Nine vertebrae are formed, of which the first is known as the cervical vertebra, or atlas, the succeeding seven are the abdominal vertebrae, and the last is called the sacral vertebra as it is to this that the pelvic girdle is attached. No caudal vertebrae are formed, but three strips of cartilage enclose the notochord and form the primordium of the adult urostyle. Dorsal ribs are differentiated, but these remain rudimentary and fuse with the transverse processes of the vertebrae. The sternum arises from the fusion of two longitudinal bars of cartilage which never attain connection with the ribs. It persists anterior and posterior to the pectoral girdle.

The cartilage bones of the skull are the exoccipitals, proötics, stapes, ethmoids, and the pterygoquadrate (in part), articulare, mentomeckelian, hyoid, and branchials. The derm bones are the fronto-parietal, nasals, premaxillae, maxillae, quadratojugals, squamosals, parasphenoid, palatines, vomers, and dentaries.

In the pectoral girdle develop the scapula, coracoid, and precoracoid, the last of which is replaced by the clavicle. In the pelvic girdle only the ilium and ischium ossify. Only four digits are present in the hand, the thumb (pollex) being absent.

THE CHICK. — There are sixteen cervical vertebrae, of which the first is the atlas, and the second, which has appropriated the centrum of the first, is the axis; five thoracic vertebrae; about six lumbar vertebrae; two sacrals; and about fifteen caudals. The last thoracic, all lumbar, and sacral and five caudals are fused to the pelvic girdle. The last four caudals are fused into a pygostyle. Dorsal ribs are formed by the cervical and the thoracic vertebrae. The sternum arises from two longitudinal bars of cartilage which unite in the median line. It is distinguished by the development of a large keel (carina) for the attachment of the pectoral muscles.

The cartilage bones of the skull are the basioccipital, exoccipitals and supra-occipitals; proötics, epiotics, and opisthotics; basisphenoid, orbitosphenoids, and alisphenoids; the ethmoid; quadrate, articular, meckelian cartilage; stapes, hyoid, and branchials. The derm bones are the frontals, parietals, nasals, lachrymals, premaxillae, maxillae, jugals, quadratojugals, squamosals, pterygoids, palatines, parasphenoids, vomer, angular, supra-angular, opercular, and dentary.

The pectoral girdle develops a scapula and coracoid, together with a dermal clavicle. Ilium, ischium, and pubis ossify separately in the pelvic girdle. Five digits are performed in the pectoral appendage; of these the first and fifth fail to develop further. Five also appear in the embryonic skeleton of the pelvic appendage; the fifth soon disappears, and the first is extremely short and develops no phalanges.

MAN. — Seven cervical vertebrae, including the axis and atlas, twelve thoracic, five lumbar, five sacral, and four caudal vertebrae are formed. Of these, the sacral vertebrae are united to the pelvis, and the caudal vertebrae are frequently fused to form the coccyx. Primordia of ribs are formed by all vertebrae except those following the first caudal. Only the thoracic segments, however, develop complete ribs. The sternum arises from two longitudinal primordia with which the first eight or nine ribs acquire cartilaginous connections.

The cartilage bones of the skull are the occipital (in part), the sphenoid, the ethmoid, the turbinates, temporals (in part), the stapes, malleus, incus, and hyoid. The malleus and incus are the representatives of the articular and quadrate. The

¹ The details of the skeleton in this and succeeding paragraphs are for reference only.

derm bones are the occipital (in part), temporals (in part), frontal, parietals, lacrymals, nasals, vomer, maxillae, zygomatics, palatines, and mandible, the last-named bone representing the fused dentaries. It is apparent that many of the bones of the human skull are the result of the fusion of separate centers of ossification which represent skull elements of the lower vertebrates. The second and third visceral arches contribute to the formation of the hyoid, the others to the laryngeal cartilage.

The pectoral girdle consists of the scapula, with which is fused the coracoid. There is no precoracoid, but a dermal clavicle is present. The centers of ossification that represent the pubis, ischium, and ilium fuse to form an innominate bone. The free appendages terminate in five digits. In conclusion, it should be mentioned that the adult condition of the human skeleton is not attained until the age of twenty-five.

G. THE MUSCLES

The musculature of the vertebrate is derived from mesenchyme (Fig. 160), of which the greater part originates from the myotomes and gives rise to striated muscle cells, controlled by the central nervous system, the skeletal musculature. A portion,

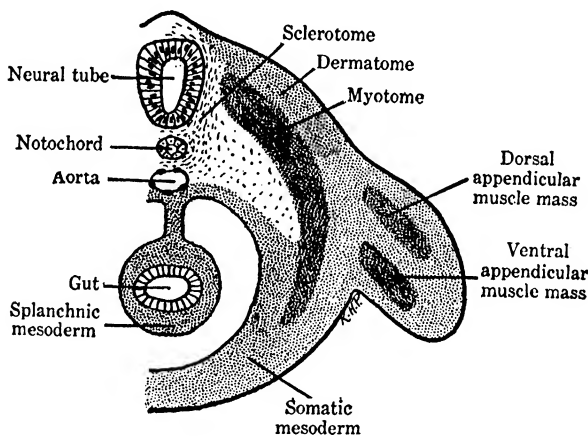


FIG. 160. — Diagram of transverse section through vertebrate embryo in region of limb bud, to show origin of appendicular muscles. (After Corning.)

however, originates from splanchnic mesoderm and gives rise to non-striated (smooth) muscle cells (found in the skin, surrounding the alimentary canal, blood vessels, and the urogenital organs. They are controlled by the autonomic nervous system (page 254), and make up the visceral musculature. Several exceptions to these general statements should be noted. The muscle cells of the heart are striated; the muscles derived from the visceral arches are both striated and controlled by the central nervous

system, although derived from lateral mesoderm. It will be noted later that the muscles of the iris of the eye (page 266) and of the sweat glands (page 246) are apparently ectodermal in origin.

Dermal musculature. — In the skin are found striped muscles which are derived from skeletal musculature (see below) but which have lost their attachment to the skeleton. The dermal musculature is best developed in the amniotes. The muscles of expression in man are dermal muscles supplied by the seventh cranial nerve (see Chapter X).

Axial musculature. — In this section are included all the muscles arising from the myotomes and attached to parts of the axial skeleton, which they move. They are originally metameric, but their later history is obscured by subsequent migration, fusion, splitting, budding, and degeneration. The intercostals, between the ribs, however, preserve their original metamorphism, which in the others may be traced to some extent by the innervation, since the connection between a spinal nerve and the muscle mass it supplies is established early in organogeny and remains constant. Thus it can be shown that the musculature of the diaphragm, supplied by the phrenic nerve, arises from a cervical myotome.

Cranial muscles. — Like the cranium, the associated muscles are derived from different sources and consist of skeletal and visceral muscles. The muscles

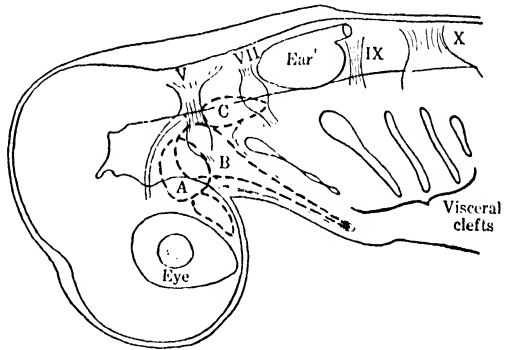


FIG. 161. — Head of embryo dogfish (*Squalus acanthias*) showing preotic somites (A, B, C) and cranial nerves (V, VII, IX, X). (After Kingsley.)

of the eyeball arise from the three preotic myotomes (Fig. 161), of which the first supplies all the muscles of the eyeball except the superior oblique, derived from the second myotome, and the lateral rectus, supplied by the third head myotome. These are innervated by the third, fourth, and sixth cranial nerves, respectively. The tongue musculature is

derived from the myotomes associated with the occipital vertebrae and supplied by the twelfth cranial nerve. The muscles of mastication, the facial muscle, and the laryngeal muscles, together with those of the ear bones, arise from the visceral arches (Fig. 162),

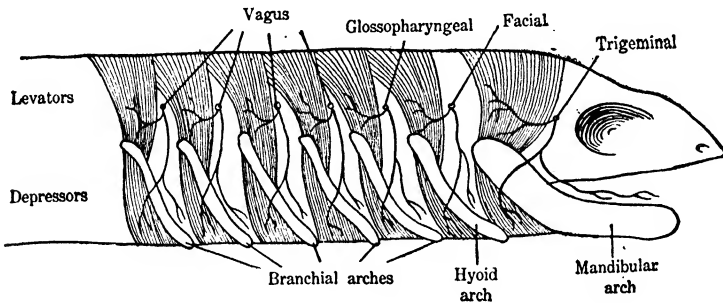


FIG. 162. — Diagram to show primitive visceral muscles in relation to visceral skeleton and cranial nerves. (Hypothetical after Wilder.)

and are supplied by cranial nerves V, VII, IX, X, and XI (see Chapter X).

Appendicular muscles. — In the anamniotes, these muscles arise from the myotomes; among the amniotes, their origin is doubtful, as the limb bud develops as an undifferentiated mass of mesenchyme surrounded by ectoderm. In this blastemal mass,

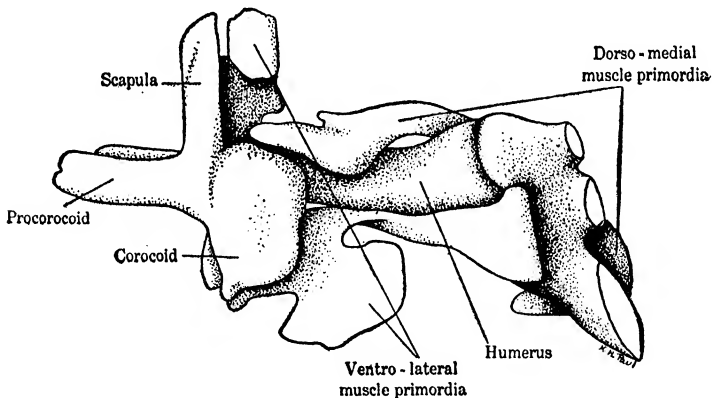


FIG. 163. — Reconstruction of the pectoral muscle masses in a 17-mm. *Necturus*. (Prepared by H. F. DeBruine.)

muscles and bones are laid down, the differentiation proceeding from the proximal toward the distal end. The pectoral muscles differentiate before those of the pelvic appendage. The appen-

dicular muscles are found in antagonistic groups: protractors, which move the limb forward; and retractors, which have the opposite effect; levators, which raise the limb; and depressors, which contract in the opposite direction. Like the axial muscles, these have become highly modified and specialized among the tetrapods (Fig. 163).

Visceral muscles. — Under this head are included the muscles lining the alimentary tract, lungs, vascular organs, and urogenital system. All arise in the mesoderm which surrounds the endothelial lining of the organs concerned. The muscle cells of the heart arise as smooth muscle cells which become striated in later development. It is interesting in this connection that the smooth muscle cells of the bladder of the dog have been transformed into what are apparently striate muscles when this organ is made to pulsate rhythmically by continued irrigation.

SUMMARY

The following structures are derived from the middle germ layer:

A. The notochord

B. The mesoderm

I. The lateral mesoderm

Epithelium of the coelom

Pericardial cavity

Pleural cavity

Peritoneal cavity

Mesenteries

Dorsal mesentery

Ventral mesentery

Mesocardia

Mesohepares

II. The intermediate mesoderm

Kidneys

Pronephros

Mesonephros

Metanephros

Genitalia

Gonads

Genital ducts

Wolffian (mesonephric) duct

Müllerian (oviducal) duct

External genitalia (also ectodermal)

Adrenal glands

Interrenals

(Suprarenals from ectoderm)

C. The mesenchyme

III. (Principally from splanchnic mesoderm)

The blood corpuscles

Blood plasma

Blood vessels

Heart

Arteries

Veins

The lymphatics

IV. (Principally from the axial mesoderm)

Connective tissue

Skeleton

Dermal

Axial

Cranial

Chondrocranium

Neurocranium

Splanchnocranium (or visceral
skeleton)

Dermocranium

Appendicular

Musculature

Dermal

Axial

Cranial

Appendicular

Visceral (from splanchnic mesoderm)

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CHAPTER X

ECTODERMAL DERIVATIVES

The ectoderm, being the external germ layer, gives rise to the outer layer of the skin, the epidermis, which continues into all the openings of the body. Of these, the development of the mouth, the cloaca and its derivatives, and the visceral clefts has been discussed. There remain for consideration the openings of the nostrils, the chamber of the eye, and the external auditory meatus. These will be taken up in connection with the sense organs, which, together with the nervous system, form in development a sensory-nervous complex.

A. THE INTEGUMENT

The integument consists of two parts, the ectodermal epidermis, and the mesodermal dermis. The epidermis soon de-

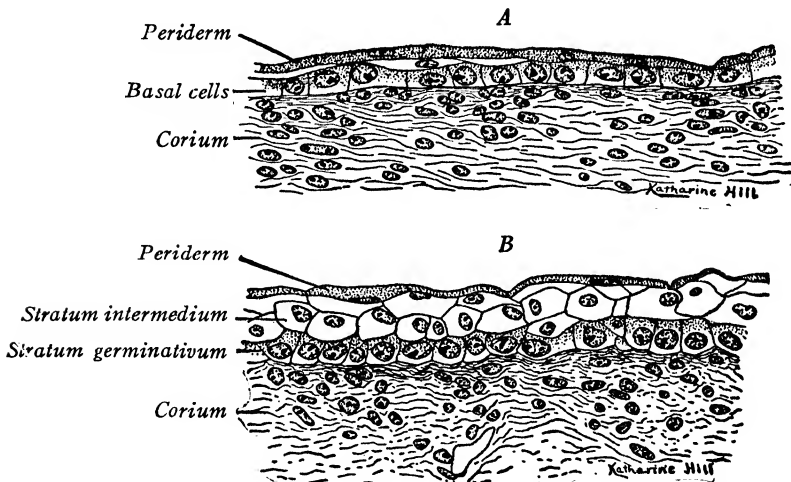


FIG. 164. — Sections through human fetal skin: A, neck. B, chin. (From Arey after Prentiss.)

laminates into two layers, the deeper germinativum, from which new strata are proliferated towards the exterior, and an outer periderm or embryonic skin (Fig. 164). Beneath the periderm,

the outer cells of the germinativum are transformed into a horny layer, the corneum. The underlying dermis is essentially a supporting layer of mesenchyme cells derived largely from the outer side of the myotome, a region which is sometimes known as the dermatome. In the dermis are formed blood vessels, connective tissue, bone, and muscle. The bony scales of fish are dermal in origin.

Derivatives of the corneum. — In the amniotes the horny layer of the epidermis is frequently fragmented to form horny scales

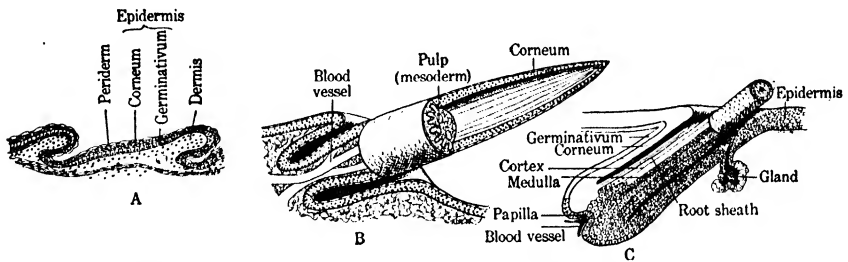


FIG. 165. — Diagrams showing similar development in A, scale; B, feather; and C, hair. (After Kingsley.)

(Fig. 165A), such as those of reptiles, or those found on the legs of birds, or the tails of rats, etc. Among the birds, scales are largely replaced by feathers which originate in much the same

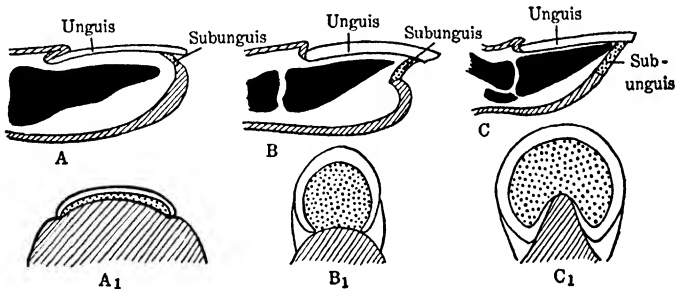


FIG. 166. — Diagrams to show ectodermal primordia of A, nail; B, claw; and C, hoof. Above in sagittal sections; below ventral view. (After Kingsley.)

manner as scales. The epidermal plate, however, grows down like a cup to enclose a core of dermal origin (Fig. 165B). The epidermal sheath gives rise to the quill and barbs, while the core gives rise to the pulp, by means of which nutriment is supplied to the developing feather. Among the mammals, hair arises in a

very similar fashion. An epidermal plate grows down into the dermis to form the hair bulb, the proximal end of which invaginates to receive a mesodermal core, the hair papilla, while around the whole is a mesodermal hair sheath (Fig. 165C). The hair papilla, however, does not grow out into the center of the hair as does the pulp of the feather. Claws, nails, and hoofs arise from the union of two epidermal primordia like those of scales, a dorsal unguis and a ventral subunguis (Fig. 166).

Derivatives of the germinativum. — The germinativum, in addition to producing the more superficial layers of the epidermis,

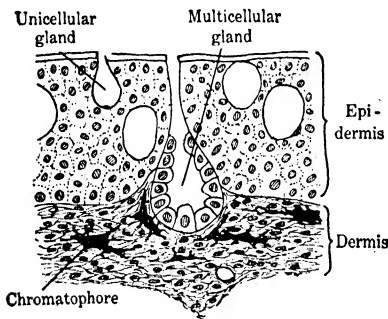


FIG. 167. — Section of *Protopterus* skin to show glands. (After Kingsley.)

gives rise to the glands of the skin (Fig. 167). Among the anamniotes, these glands are usually unicellular and produce the mucus which serves to diminish the friction of the skin against the water while swimming. Unicellular glands frequently aggregate to produce multicellular glands, such as the flask glands and cement glands of the anamniotes, or the sebaceous (oil) and sudoriparous (sweat) glands of the mammals.

The mammary glands of mammals are modified sudoriparous glands secreting the milk by which the new born are nourished through infancy.

Derivatives of the dermis. — Two types of pigmentation are to be distinguished in the integument. The first is produced by pigment secreted in the ectodermal epidermis, i.e., the melanin, of the frog tadpole. The second is produced by chromatophores, which are mesenchyme cells of the dermis. These secrete pigment granules and move toward the light to form a layer immediately below the epidermis, some even wandering into the epidermis itself.

THE FROG. — The ectoderm of the frog embryo is ciliated at 6-mm. body length and remains so until the length of 20 mm. is attained, when the cilia disappear except on the tail which remains ciliated until metamorphosis. The jaws and oral combs of the tadpole are derivatives of the corneum and consist of rows

of horny denticles forming replacement series. The oral gland, or sucker, is a multicellular mucous gland derived from the germinativum and elevated by the elongation of its gland cells. It arises as a crescentic groove posterior and ventral to the point where the stomodeum will appear, then becomes V-shaped, and finally divides by the degeneration of the middle portion. The cement gland atrophies soon after the opening of the mouth. The pigmentation of the skin is derived from two sources, the melanin of the egg which is distributed to the epidermis, and the mesenchymal chromatophores (Fig. 199) which develop in the dermis.

THE CHICK. — The scales on the legs are typical reptilian scales and are derived from the corneum; they sometimes bear feathers in the young bird and so form a transition between scales and the characteristic avian feathers. The claws arise in the corneum from two primordia, a dorsal "claw-plate" and a softer "claw-sole." To prevent the sharp claws tearing the embryonic membranes, the concavity of the claw is filled with a pad known as the neonychium, derived from the corneum, which is lost after hatching. The beak arises from the corneum around the upper and lower margins of the jaws. The egg tooth is a horny prominence on the dorsal side of the upper jaw, appearing on the sixth day of incubation but not taking on its ultimate shape until the fourteenth. It serves to aid in breaking the shell and is lost after hatching.

MAN. — The nails arise from nail-plates and sole-plates, of which the latter are rudimentary structures. They are covered during fetal life by the eponychium, consisting of the periderm and outer layers of the corneum. The hairs are arranged in patterns which have been interpreted as reminiscences of the ancestral scales. The first growth of hair is called the lanugo; it is cast off, except over the face, soon after birth. The mammary glands arise from two longitudinal thickenings of the epidermis, known as the milk ridge. In later development the gland resembles an aggregation of sudoriparous glands.

B. THE NERVOUS SYSTEM

Although the nervous system and sense organs arise together and remain in functional continuity, it has become customary to distinguish the sense organs (receptors) from the nerves (trans-

mittors) by which stimuli are passed on to the muscles or glands (effectors). Both the nervous system and the sense organs arise from specialized regions of the dorsal ectoderm, known respectively as the neural plate and the sense plates (placodes). These represent an inward growth from the germinativum as opposed to the outward growth which produces the epidermis. In the frog this division is clearly indicated by a line of cleavage between the outer epidermal ectoderm and the inner nervous ectoderm. Both the neural plate and the sensory placodes withdraw from the surface and become subepidermal by a process of invagination. In this connection it is interesting to note that the optic placode is incorporated and invaginates with the neural plate so that when the retina of the eye develops, it does so from the brain.

The neural tube. — The neural plate is an elongate structure, extending from the blastopore to the head region. Local growth results in the incurving of this plate to produce a neural groove with conspicuous lips, the neural folds. As this growth continues the groove sinks inward and the lips meet above it, thus converting the groove into a neural tube, which breaks away from the overlying epidermis and sinks into the interior. The cells at the margin of the neural plate form, at each dorso-lateral angle of the neural tube, a bar known as the neural crest, which subsequently segments into the ganglia.

The neurons. — The inner lining of the neural tube, corresponding to the outer layer of the neural plate, is called the ependyma. This is the center of cell proliferation (Fig. 170). Two types of cells are formed: the supporting cells, or spongioblasts; and the embryonic nerve cells, or neuroblasts. The neuroblasts migrate out of the ependyma and form an intermediate mantle layer in which they become transformed into neurons. These nerve elements have a prolongation at one end known as the axon or nerve fiber, while at the other are branched projections called dendrites. The axons grow out from the mantle layer into the outer layer of the cord, known as the marginal layer, where they secrete the medullary sheaths which act as insulating coats. Not all axons become medullated. Similar changes take place in the ganglia, whereby neurons and supporting cells are differentiated.

Types of neurons. — We may distinguish four types of neurons (Fig. 168), as follows: (1) Afferent neurons arising in the ganglia and sending their axons to the dorsal region of the neural tube. These convey excitations from the sensory receptors to the neural tube. Two sub-types are distinguished: (a) the somatic sensory neurons, conveying excitations from the exterior; and (b) splanchnic sensory neurons, conveying excitations from the viscera. (2) Efferent neurons, with their bodies in the ventral region of the neural tube, sending their axons to effectors (muscles or glands). Two sub-types are recognized: (a) somatic motor and (b) splanchnic motor. These afferent and efferent neurons form the peripheral nervous system. (3) The intersegmental neurons have their bodies in the ventral portion of the neural tube and their axons are usually directed towards its posterior end. They serve to connect efferent neurons in the different segments of the body. (4) The suprasegmental neurons have their bodies usually in the dorsal portion of the neural tube and their axons are directed toward the anterior end of the tube, i.e., the brain. They serve to convey afferent excitations toward the brain and in that organ give rise to the great brain centers. The axons of

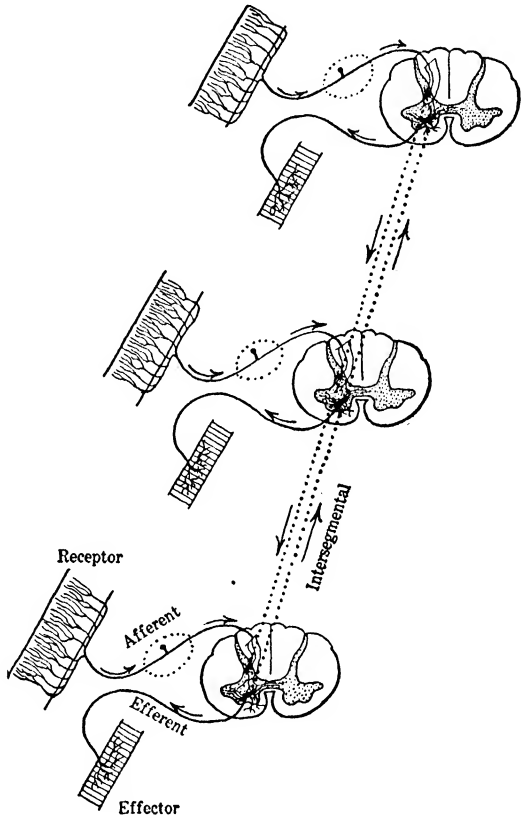


FIG. 168. — Diagram to show cross-sections of the spinal cord at three levels, the posterior level above. The dotted lines indicate the paths of neurons whose bodies lie wholly within the cord, suprasegmental to the left.

these last two types of neurons form the descending and ascending bundles of the brain and cord.

The spinal cord. — The spinal cord, or neural tube exclusive of the brain, retains its primitive characteristics. The cavity, or neurocoel, persists as the central canal. (Between each pair of vertebrae the afferent and efferent neurons form a pair of spinal nerves which run out into the myotomes and hence have a metamerism equivalent to that of the myotomes, an important point in considering the homologies of the muscles. In the region of the pectoral and pelvic appendages, several of the segmental nerves combine to form the brachial and the sacral plexus, respectively. The cord becomes surrounded by an envelope of mesenchyme known as the meninx, which in the higher vertebrates becomes divided into an inner pia mater and an outer dura mater. The development of the nerves will be taken up in a later section.

The brain. — Whereas the cord is largely composed of afferent, efferent, and intersegmental neurons, by which certain reflex actions are directed, the anterior end of the neural tube enlarges and differentiates into the complex brain (Fig. 169). Here arise several centers in which the impulses received mainly from the major sense organs, nose, eye, and ear, are correlated. The brain may be divided into two major regions: the archencephalon, or prechordal brain; and the deutencephalon, or epichordal brain. With continued local growth, the archencephalon grows down in front of the notochord, thus forming the first or cranial flexure. At the same time, three dilations appear: the prosencephalon from the archencephalon; the mesencephalon at the point of the flexure; and the rhombencephalon from the deutencephalon.) It is convenient to associate the future history of the prosencephalon with that of the nose, the mesencephalon with that of the eye, and the rhombencephalon with that of the ear.

The prosencephalon. — The later history of the prosencephalon is complicated by the fact that the optic placode is included in the neural tube at this point. Accordingly, we find the prosencephalon dividing into an anterior telencephalon and a posterior diencephalon.

The telencephalon. — The anterior part of the telencephalon becomes the olfactory lobe, which receives the afferent neurons from the nose. From the roof develops the cerebrum, which be-

comes the most complex and important center of association. From the floor arises the optic part of the hypothalamus. There are two cavities, or telocoels (also known as the lateral ventricles).

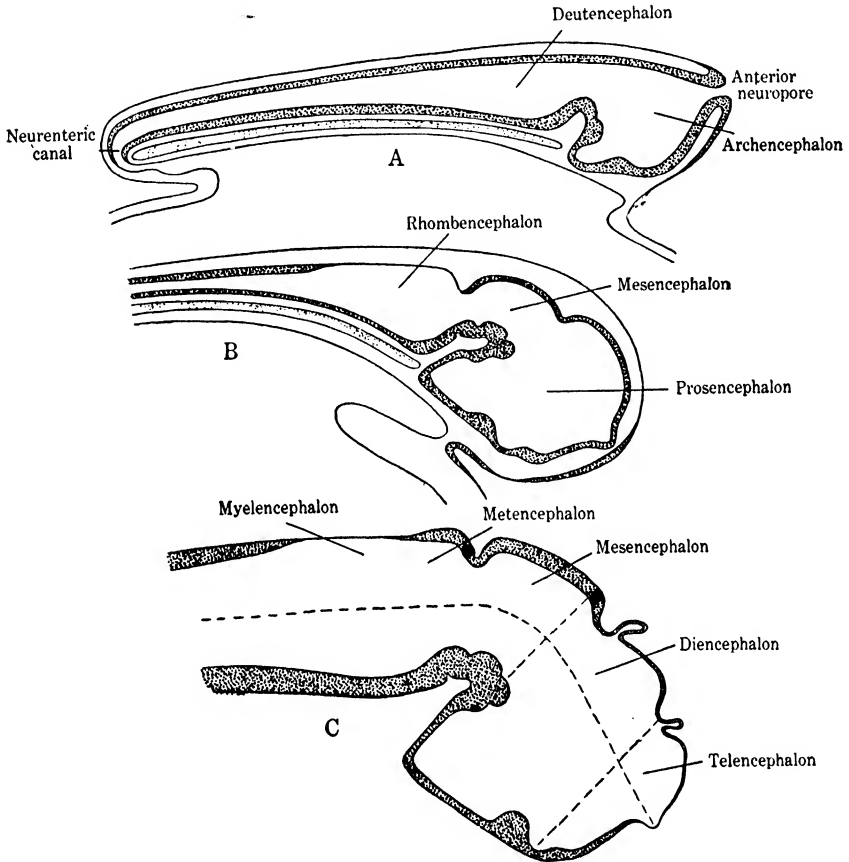


FIG. 169. — Diagrams to show early development of the vertebrate brain in sagittal sections. A, prechordal and epichordal divisions. B, primary brain vesicles. C, definitive vesicles. The longitudinal broken line indicates division between roof and floor regions. (After von Kuppfer.)

The diencephalon. — The roof of the diencephalon gives rise to the thalamus in front, and the metathalamus behind; from the latter springs a dorsal diverticulum, the epithalamus. This structure, often known as the epiphysis, gives rise to something very much resembling an unpaired eye in early embryonic life; this later becomes the pineal gland of the adult, one of the so-

called endocrine glands. /The eyes take their origin from the side of the diencephalon. The floor of the diencephalon gives rise to a ventral diverticulum — the infundibulum, which grows downward to meet the advancing hypophysis from the stomodeum (see page 181). The two later fuse to form the pituitary gland, another of the endocrine series. Behind the infundibulum, the floor of the diencephalon forms the mammillary part of the hypothalamus. It is evident that the thalamencephalon, often used as a synonym of the diencephalon, differs from it by the inclusion of the optic part of the hypothalamus, which is derived from the telencephalon although indistinguishable from the mammillary part of the hypothalamus in the adult. The thalami contain nuclei (masses of neurons) which receive afferent impulses from the optic, general sensory, and acoustic organs, and transmit impulses to and from the other centers of the brain. The cavity of the diencephalon persists as the diacoel (third ventricle).

The mesencephalon. — The roof of the mesencephalon gives rise to the corpora bigemina (quadrigemina in mammals), or optic lobes, the centers which receive afferent impulses from the eyes transmitted through the diencephalon. The floor of the mesencephalon is the anterior portion of the brain stem, from which the motor neurons of the cranial nerves depart. The third and fourth cranial nerves originate from the mesencephalon. Its cavity is the mesocoel (or aqueduct).

The rhombencephalon. — The hind-brain, like the fore-brain, is divided into two regions, metencephalon and myelencephalon, respectively.

The metencephalon. — The roof of the metencephalon gives rise to the cerebellum, the center associated with hearing (except in mammals), the lateral line organs of anamniotes, and the sense of equilibrium. The floor of the metencephalon is part of the brain stem, and from it arises the pons, a bundle of axons connecting the two sides of the cerebellum. The cavity is the metacoel.

The myelencephalon. — The roof of the myelencephalon is covered by a thin roof plate, the choroid plexus. Its floor forms the posterior portion of the brain stem. (The cranial nerves, from V to XII inclusive, depart from this portion of the stem, which merges imperceptibly into the spinal cord. Its cavity,

hardly distinguishable from that of the metencephalon, is called the myelocoel (~~fourth~~ ventricle).

The spinal nerves. — (The nerves are segmentally arranged bundles of afferent and efferent neurons originally associated with the myotomes. The afferent neurons arise in the ganglia, the efferent in the floor of the spinal cord. Accordingly, a typical spinal nerve has two roots in the cord: a dorsal afferent root uniting with the ganglion; and a ventral efferent root which unites with the dorsal root after the other has attached itself to the ganglion (Fig. 170). The nerve trunk then divides into branches, each containing afferent and efferent neurons, which are called *rami* and supply the body wall, although one (the com-

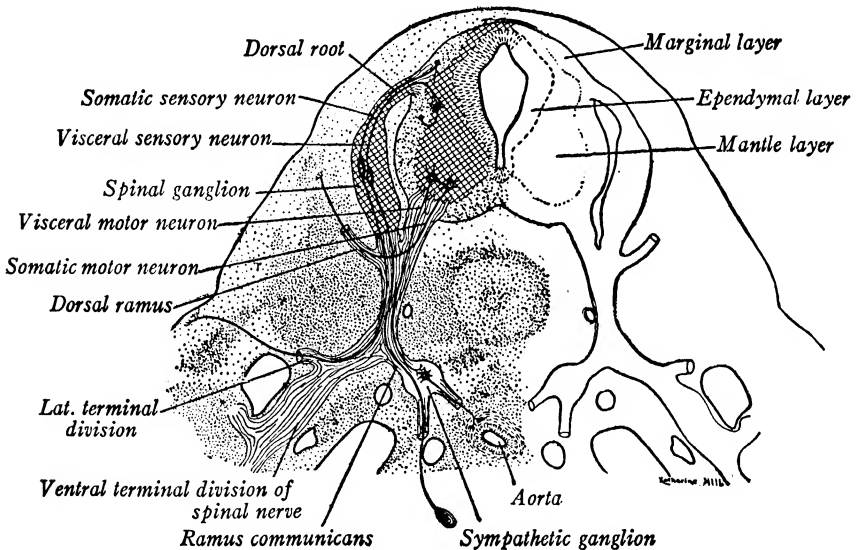


FIG. 170. — Diagram to show the neuron components of a spinal nerve. Transverse section of 10 mm. human embryo. (From Arey after Prentiss.)

municating ramus) connects with a sympathetic ganglion, derived from a spinal ganglion, through which the splanchnic afferent and efferent neurons serve the viscera.

It has been shown by Coghill that the development of behavior is closely paralleled by the development of the connections (synapses) between the neurons. Thus in the urodele, *Ambystoma*, the first reflex of the embryo, a bending away from a light touch on the skin, does not take place until an intermediate

neuron in the spinal cord has established synaptic relations with the sensory tract on one hand and a floor plate cell which already has established a synaptic relation to the motor tract on the opposite side of the spinal cord (Fig. 171).

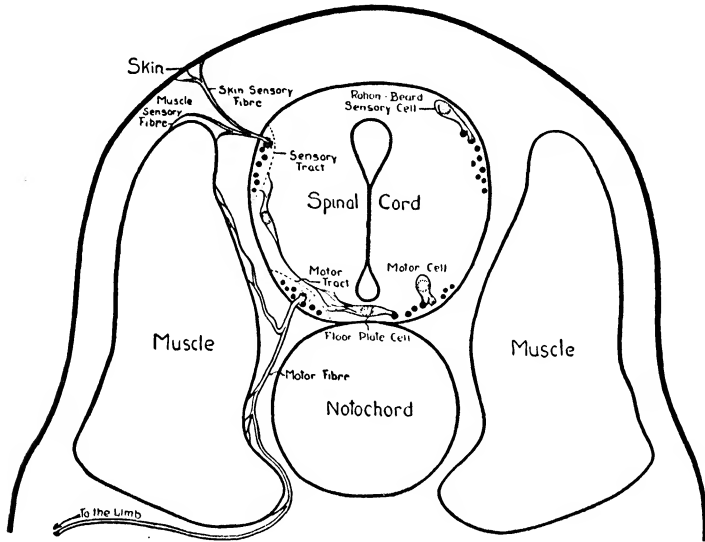


FIG. 171. — Diagram to show in transverse section of *Ambystoma* larva, neurons concerned in earliest reflex. (From Coghill, "Anatomy and the Problem of Behavior.")

The autonomic nerves. — The brain, spinal cord, and cranial and spinal nerves are grouped by anatomists as the central nervous system. Associated with this is the autonomic nervous system, consisting of nerves and ganglia and controlling the smooth muscles of the viscera and blood vessels, and some glands. This system arises from the neural plate, like the central nervous system, but from the lateral margins which become the neural crests. At the time when the neural crests are dividing into the cerebro-spinal ganglia, some of the cells migrate inward toward the dorsal aorta, where they aggregate and multiply to form the chain ganglia. The chain ganglia on each side become connected by fore and aft extensions which form the sympathetic trunks. They retain a connection with the cranial and spinal ganglia by means of the communicating rami, and send out nerves along the principal blood vessels. From the chain ganglia, by secondary and tertiary

migrations, arise the prevertebral and visceral ganglia. In the head the four sympathetic ganglia (ciliary, sphenopalatine, otic, and submaxillary)

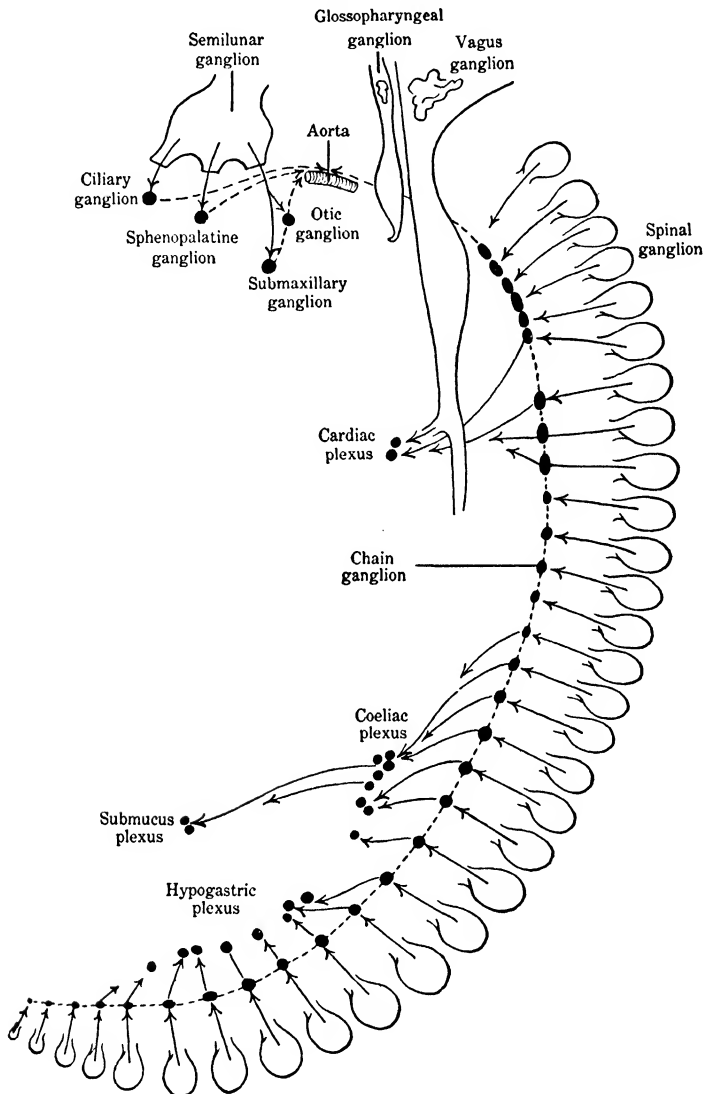


FIG. 172. — Diagram to show migrations of autonomic ganglia in human development. (After Streeter.)

and submaxillary) arise from the semilunar ganglion of the fifth cranial nerve, and later acquire connections with the chain ganglia (Fig. 172).

It has already been noted (page 214) that some of the cells from the autonomic ganglia (chromaffin cells) migrate to the vicinity of the mesonephros to form the suprarenal gland.

The cranial nerves.—The cranial nerves, or nerves of the head regions, contain not only splanchnic and somatic afferent

and efferent neurons comparable to those of the spinal nerves, but also special afferent neurons from the nose, eye, ear and lateral line system. There are ten cranial nerves in the anamniotes, twelve in the amniotes (Figs. 173, 174). To these should be added in all cases the terminal nerve, unknown when the cranial nerves were first classified.

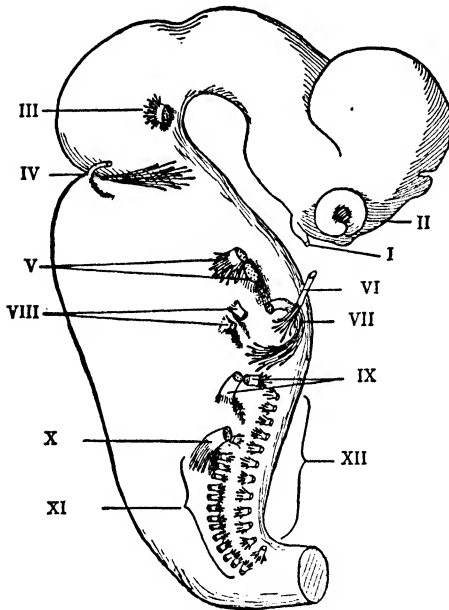


FIG. 173. — Diagram to show origin of cranial nerves in man. (After His.)

O. Terminal, a ganglionated nerve from the organ of Jacobson entering the cerebral lobe with functions unknown, probably sensory.

I. Olfactory, a non-ganglionated sensory nerve from the olfactory sensory region

of the nose to the olfactory lobe.

II. Optic (ophthalmic), a non-ganglionated sensory nerve from the retina of the eye to the floor of the diencephalon where the fibers from the two eyes cross (optic chiasma). Each set of fibers then enters the brain and runs to the optic lobe on the opposite side of the brain to that on which the eye is located.

III. Oculomotor (motor oculi), a motor nerve, somatic with some sensory elements, from the floor of the mesencephalon to all muscles of the eyeball except the superior oblique and the lateral rectus.

IV. Trochlear, a motor nerve, somatic with some sensory elements, from the roof of the mid-brain to the superior oblique muscle of the eyeball.

V. Trigeminal, a mixed nerve. Its somatic sensory neurons arise in the semilunar ganglion, the motor elements in the floor of the myelencephalon. The sensory neurons are somatic (general cutaneous). The motor neurons supply the jaws (mandibular arch).

VI. Abducens (pathetic), a somatic motor nerve with some sensory elements, arising from the myelencephalon and supplying the external rectus muscle of the eyeball.

VII. Facial, a mixed nerve. The afferent neurons arise in the geniculate ganglion and are splanchnic in nature, supplying the

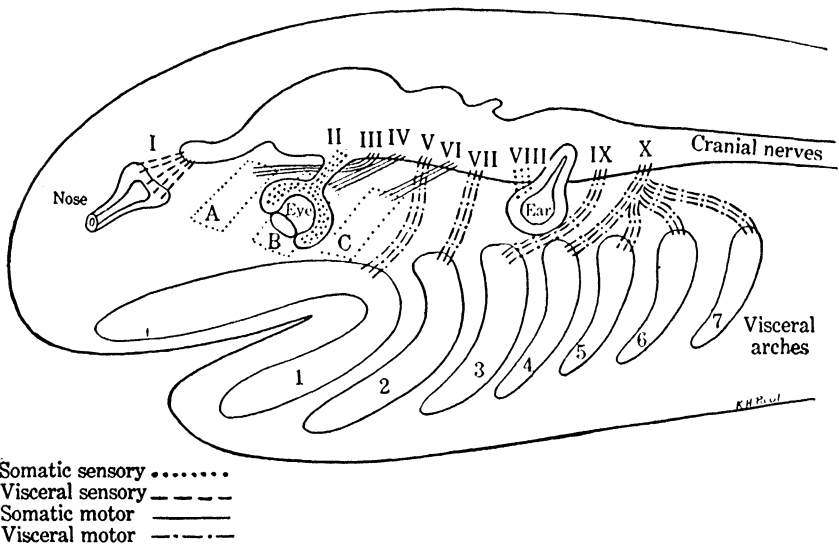


FIG. 174. — Diagram showing relationships between cranial nerves and parts supplied. A, B, C, head somites. Arabic numerals, visceral arches. Roman numerals, nerves.

hyoid arch, and also the tongue of mammals. In the anamniotes, an associated ganglion gives rise to a lateral branch with afferent components from the lateral line organs. The efferent neurons supply the hyoid arch in the lower vertebrates and the facial region in the amniotes. The rami of the fifth and seventh nerves are closely associated.

VIII. Acoustic (auditory), a ganglionated sensory nerve arising from the acoustic ganglion and bearing afferent neurons from the ear. In higher vertebrates it becomes differentiated into the

vestibular and cochlear nerves, each with its own ganglion produced by the division of the acoustic ganglion.

IX. Glossopharyngeal, a mixed nerve. The afferent neurons arise in the petrosal and the superior ganglion and are principally splanchnic. They divide into a prebranchial branch running into the hyoid arch and a postbranchial branch into the first branchial arch. The efferent components are principally found in the postbranchial branch.

X. Vagus, a mixed nerve arising by the fusion of several primitive cranial nerves, which supplied the arches with afferent (from the jugular ganglion) and efferent neurons. In addition, the vagus gives off a visceral branch to the stomach, lungs, etc., and in the anamniotes a lateral branch to the lateral line organs of the trunk (from the nodosum ganglion).

XI. Accessory, a motor nerve which innervates the muscles of the shoulder girdle and is found only in the amniotes. A ganglion (of Froriep) disappears before the embryo becomes adult.

XII. Hypoglossal, also a motor nerve, which innervates the tongue in the amniotes. In the anamniotes the tongue is innervated by so-called "occipital" nerves which possibly are the fore-runners of the hypoglossal, prior to the appropriation of the occipital region by the head.

Metamerism of the nervous system.—The metameric arrangement of the nerves, like that of the segmental arteries, is purely secondary and dependent upon the primary metamerism of the mesoderm. The nerves, however, are more conservative than the vascular organs or myotomic derivatives. For example, the diaphragm of mammals is supplied by muscles from one of the cervical myotomes, and the innervation of the diaphragm (phrenic nerve) still arises from the cervical region. Many attempts have been made to reconstruct the metamerism of the head, by a study of the cranial nerves, following Bell's law: that every original cranial nerve has, like a spinal nerve, a dorsal sensory and ventral motor root.

This problem has been complicated by the fact that in the head there are two types of metamerism, (1) primary as indicated by the head myotomes in the elasmobranch embryo, and (2) secondary (branchiomic) as indicated by the visceral arches (Fig. 174). Accordingly, there are two types of musculature,

TABLE 11
NEURONE COMPONENTS OF CRANIAL NERVES AND FUNCTIONS

Nerve	Afferent Somatic	Afferent Splanchnic	Efferent Somatic	Efferent Splanchnic
I		Smell		
II	Vision			
III			Movement of eyeball	
IV			Movement of eyeball	
V	General cutaneous			Movement of jaw
VI			Movement of eyeball	
VII		Taste		Hyoid and facial movement and salivation
VIII	Hearing and equilibration			
IX		Taste and pharyngeal sensation		Salivation, pharyngeal movement
X		Visceral sensation		Movement of viscera and pharynx
XI				Movement of pharynx and shoulder
XII			Movement of tongue	

(1) somatic as represented by the muscles of the eyeball, and (2) splanchnic as represented by the muscles of the jaws and visceral arches. Two types of efferent neurons, therefore, are present, (1) somatic and (2) splanchnic. The splanchnic motor neurons of the cranial nerves differ from those of the trunk, however, in that no sympathetic neurons intervene between them and the muscles which they supply. There are altogether three sets of afferent neurons: (1) the general sensory or cutaneous, which correspond to the somatic sensory neurons of the trunk; (2) splanchnic sensory, which correspond to those of the trunk; and (3) lateral, belonging to the lateral line system. The cranial nerves are evidently not serially homologous, as can be seen from Table 11.

Finally, we must mention the neuromeres which have been reported in various vertebrate embryos. These are formed by constrictions in the cranial portion of the neural tube and interpreted by some authors as the remains of a neural metamerism. They seem in many forms to correspond with the cranial nerves and more probably represent areas of local growth prior to the outgrowth of the nerves themselves.

The general problem of the metamerism of the head still awaits solution. The latest summary, that of Brachet, indicates the probable number of segments in the primitive head as six. Three of these are ephemeral, and their somites give rise to mesenchyme. The three posterior segments are associated with the first three visceral clefts bounded by the first four arches, each of which has its own cranial nerve: the trigeminal of the mandibular arch; the facial of the hyoid; the glossopharyngeal of the first branchial; the vagus of the second branchial arch. According to this interpretation, the posterior clefts and arches are reduplications supplied by new branches of the vagus, while the accessory and hypoglossal are secondarily acquired spinal nerves.

THE FROG (SEE ALSO CHAPTER XI). — The prechordal and epichordal divisions of the brain are demarcated by the notochord, and the division into the three primary vesicles is but slightly indicated. The brain of the frog never develops neuromeres. The optic lobes are corpora bigemina. The division into myelencephalon and metencephalon is incomplete, and no pons is formed. There are forty pairs of spinal nerves in the tadpole, reduced to

ten in the adult. There are but ten of the cranial nerves (XI and XII not included). The sympathetic ganglia originate from the cranial and spinal ganglia by the emigration of ganglion cells.

THE CHICK (SEE ALSO CHAPTER XII). — The divisions of the brain into the three primary and five secondary vesicles is well marked. Eleven neuromeres are formed, of which three are found in the prosencephalon, two in the mesencephalon, the remainder in the rhombencephalon. Three flexures are formed: (1) cranial in the floor of the mesencephalon; (2) cervical at the junction of the myelencephalon and the spinal cord; and (3) pontine in the floor of the myelencephalon. A pons is formed. There are fifty pairs of nerves developed in the chick of eight days (Lillie), of which thirty-eight are spinal and twelve cranial, including the eleventh and twelfth which are not incorporated in the head of the frog.

MAN (SEE ALSO CHAPTER XIII). — The particular feature of importance in the development of the human brain is the great increase in size and complexity of the cerebral hemispheres of the telencephalon. The optic lobes are quadripartite (*corpora quadrigemina*), of which the two anterior lobes are especially associated with vision, the two posterior ones with hearing.

C. THE SENSE ORGANS

The nervous system receives stimuli not only from outside the body but also from within, such as those concerning the tension of the muscles. For the reception of stimuli, special organs — the sense organs — are developed. Of these the most conspicuous are the eyes, the ears, and the nose. In addition, it must be remembered that the entire skin functions as a sense organ by means of special receptors, and that stimuli are received from the viscera and other internal structures by means of free nerve terminations.

Of the special sense organs, the eye is most specialized in its mode of development. It is responsive to photic stimuli. The nose represents a concentration of chemical sense receptors, more highly developed than the scattered taste buds of the head, which are confined in adult mammals to the cavity of the mouth. The ear, responsive to slower vibrations (pressure, sound) in the surrounding medium, originates in a manner similar to that of

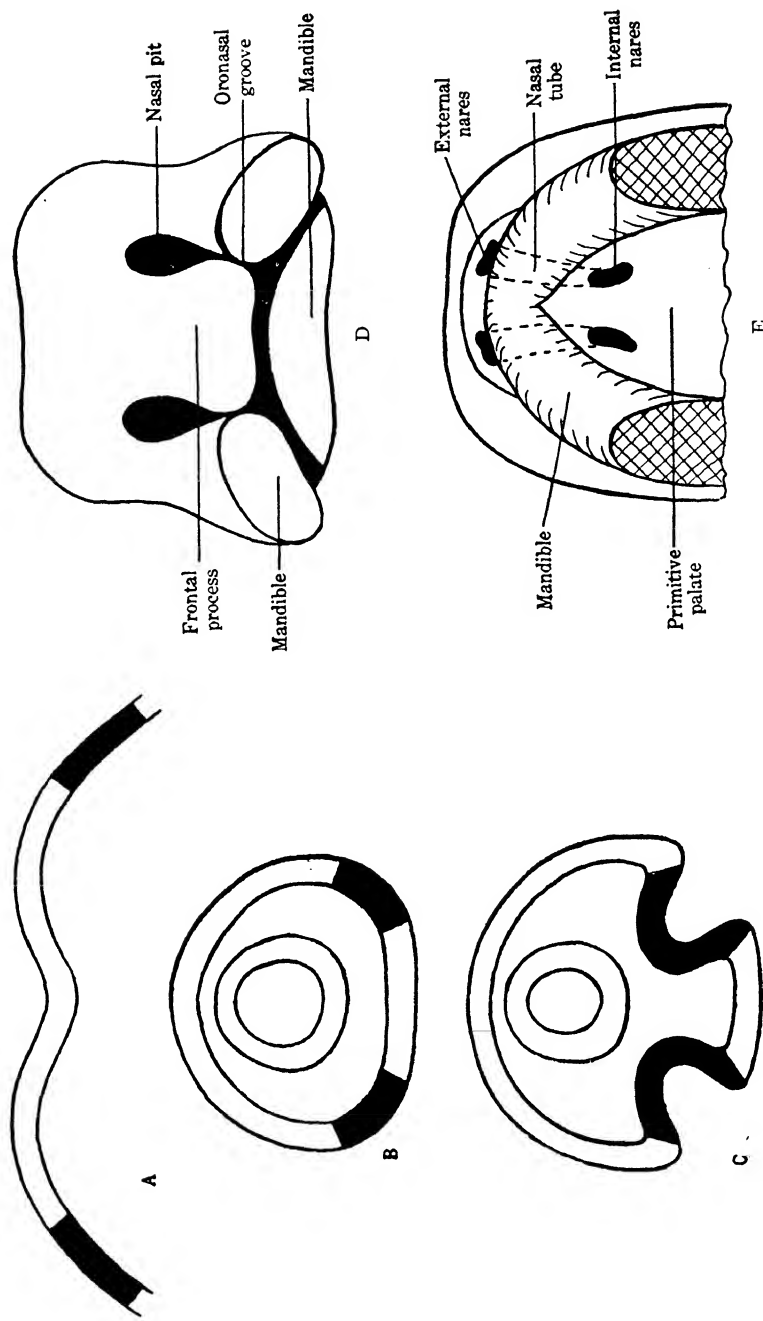


FIG. 175. — Diagrams showing early stages in development of nose. A, nasal placodes (in black). B, same now on ventral surface of head. C, nasal pits. D, nasal pits. E, nasal tubes, ventral view, lower jaw removed.

the lateral line system. This system is highly developed in the aquatic anamniotes, vestigial or absent in the amniotes. The ear, on the other hand, is more highly developed in the amniotes.

The nose. — The nose arises as a pair of local thickenings of the ectoderm at the anterior end of the head (Fig. 175). These thickenings are hereafter known as the nasal (olfactory) placodes. Later they invaginate to form the nasal (olfactory) pits, which persist as the nose of all fish except the air-breathing dipnoi. Here also should be noted the fact that the cyclostomes are peculiar in the possession of a single median nasal pit. Among the tetrapods (the nasal pits elongate to become oro-nasal grooves, the anterior ends of which become connected with the developing mouth into which they are carried.) The original anterior ends

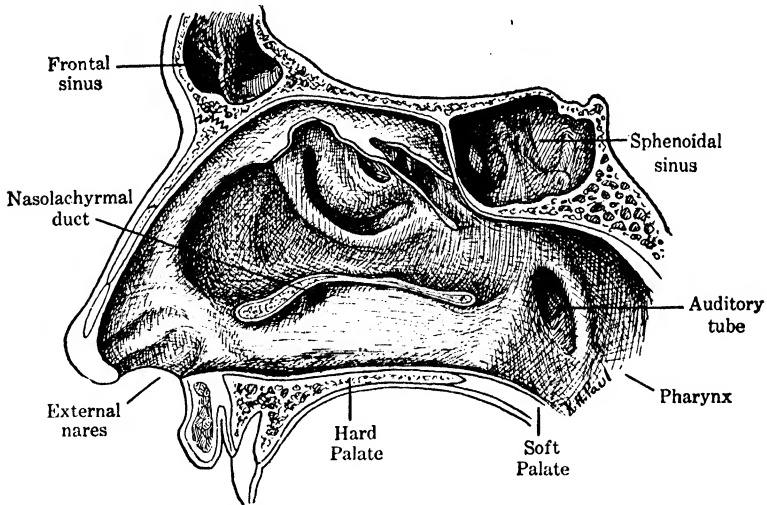


FIG. 176. — Sagittal hemi-section through human nose. (After Howden.)

of the nasal pits, therefore, come to lie at the posterior end of the mouth and open into the pharynx as the internal nares, while the original posterior ends become the external nares (Fig. 175E). The nasal cavity is later separated from the oral cavity by the ingrowth of the maxillary, palatine, and pterygoid bones, which form the hard palate (Fig. 176). Jacobson's organ arises as a pocket of the olfactory epithelium. Its function is unknown. The olfactory epithelium contains ciliated cells connected to the olfactory lobe by means of the first cranial nerve) which is

peculiar in that its neurons run directly to the brain without the interposition of ganglion cells. Jacobson's organ receives a branch of the trigeminal nerve.

The eye. — The optic placodes are incorporated into the neural plate, where they can be distinguished as lateral thickenings of the margin at points which will later be included in the diencephalon (Fig. 177). When the tube is formed, the relation of the sensory epithelial cells to the exterior is, of course, reversed. The optic placodes "invaginate," but, owing to their relation to the neural tube, the result is an apparent "evagination" from the tube towards the exterior. This produces the outgrowths which later, by constriction, give rise to the proximal optic stalks and distal optic vesicles. At the point where the optic vesicle touches the ectoderm, two reactions take place: (1) a local thickening of the ectoderm, called the lens placode, from which the lens of the eye develops; and (2) an invagination of the optic vesicle whereby this vesicle is transformed into a two-layered optic cup. This invagination continues into the optic stalk to produce a groove called the choroid fissure.

The lens. — (The lens placode invaginates to form the lens pit, which then withdraws still further from the surface and becomes closed in by the union of its external lip to form the lens vesicle. The lens vesicle becomes solid by the elongation of the cells on the internal side which assume a clear transparent appearance.)

The optic cup. — The inner layer of the cup becomes the sensory portion of the retina, the outer layer the pigmented portion. It will be recalled that the sensory epithelium of the eye is inverted, and as a result the rods and cones, or sensory elements, of the retina are pointed away from the light. In the pigmented layer of the retina, melanin is secreted. Meantime the cavity of the optic cup becomes filled with a clear fluid secreted by the surrounding cells, which later becomes viscous and forms the vitreous humor.)

The envelopes of the eyeball (Fig. 178). — Around the optic cup and stalk, a layer of mesenchyme accumulates, which later differentiates into an inner delicate layer called the choroid which contains pigment and capillaries and may be compared with the pia mater of the brain, and an outer dense layer known as the sclera, which may be compared with the dura mater of the brain.

The external portion of the sclera over the lens makes contact with the epidermis and becomes transparent to form the cornea.

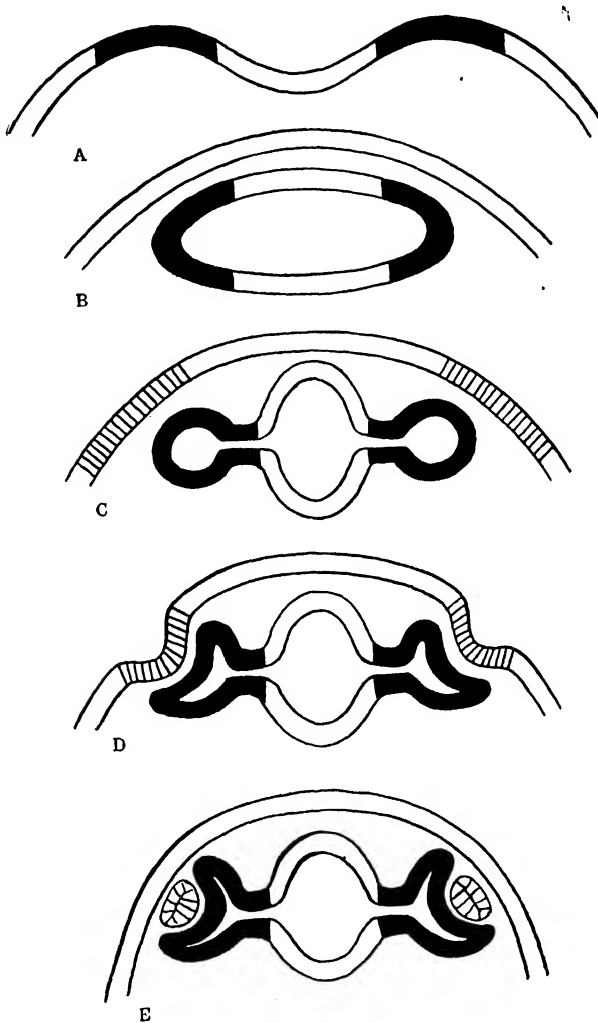


FIG. 177. — Diagrams showing early stages in development of vertebrate eye. A, optic placodes (in black). B, same after formation of neural tube. C, optic vesicles and lens placodes. D, optic cups and lens pits. E, optic cups and lens vesicles.

The epidermis over the eye forms the conjunctiva. In some vertebrates, sclerotic cartilage, or even bone, is formed, the vestige of an optic capsule. The edge of the choroid, together with

the marginal retina, gives rise to the iris, a circular curtain surrounding the opening of the cup which is called the pupil of the eye. The muscles of the iris are apparently of ectodermal origin. The iris divides the space between the lens and the cornea into two chambers, an anterior and a posterior chamber, which are filled with a fluid, the aqueous humor. The muscles of the

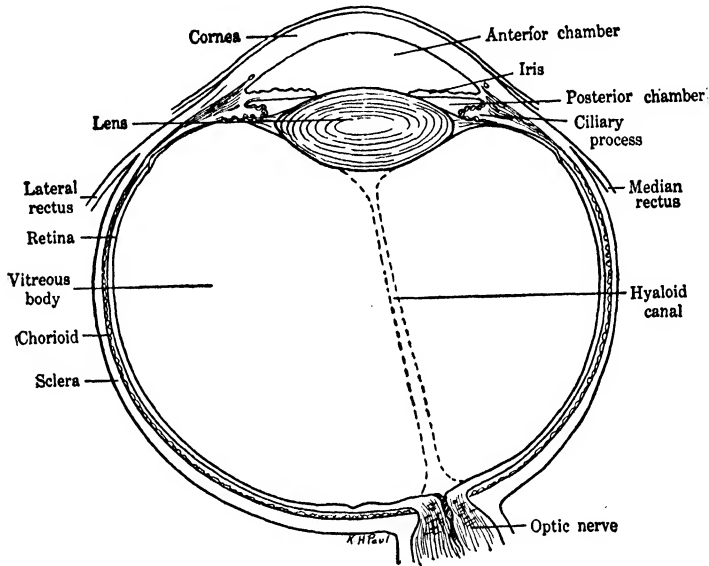


FIG. 178. — Horizontal section of human eye. (After Howden.)

eyeball are six in number, arising from the three head myotomes. They are innervated by the oculomotor, trochlear, and abducens nerves.

The optic nerve. — The afferent neurons pass from the retina into the optic cup and form a bundle which passes out through the choroid fissure and into the optic stalk, and so to the optic chiasma on the floor of the diencephalon, where they cross and make their way to the optic lobes on the opposite side.

The lateral line system. — This is a diffuse sensory organ consisting of sense buds arranged in rows over the head and body of aquatic anamniotes. Its function apparently is to detect slow vibrations in the water. The origin of the lateral line system is a lateral thickening of the sensory ectoderm which later breaks up into separate suprabranchial placodes. These are found in the

embryos of the amniotes but soon degenerate. The lateral line system is of particular interest inasmuch as the lateral thickening referred to is in some cases continuous with the otic placode which gives rise to the ear. The principal nerve supplying the lateral system is the facial, although trigeminal, glossopharyngeal, and vagus often contain lateral line components.

The ear. — The ear becomes differentiated into the vestibule or equilibratory organ and the cochlea or organ of hearing. Three parts of the ear are distinguished (Fig. 180). The inner ear, giving rise to the vestibule and the cochlea, arises from an ectodermal otic (auditory) placode. The middle ear appears in the amphibians, and it is derived from the endodermal first visceral pouch. The outer ear, found only in the amniotes, is an ectodermal derivative of the first visceral groove and an outgrowth from the mandibular and hyoid arches.)

The inner ear. — This originates from the otic placode, which invaginates to form an otic (auditory) pit (Fig. 179) and later closes over to withdraw from the epidermis as the otic (auditory) vesicle or otocyst. In some vertebrates (elasmobranchs) the vesicle retains its connection with the exterior by means of a hollow stalk, the endolymphatic duct. Usually this connection is lost and the endolymph duct of the adult is a new formation. The vesicle divides into a ventral saccule and a dorsal vestibule or utricle. The saccule gives rise to the endolymph duct and the lagena, which in mammals becomes the coiled cochlea or organ of hearing, while the utricle gives rise by constriction to three semi-circular canals, each with a dilation at one end, the ampulla. The sensory epithelium is restricted to the lagena and ampullae. The cavity of these structures is known as the membranous labyrinth, and contains a fluid, the endolymph. Concretions, the otoliths, may appear in the endolymph of the vestibular portion. Around this labyrinth the otic capsule, or skeletal labyrinth is formed. This later ossifies to give rise to the otic bones. (The skeletal labyrinth contains a fluid known as the perilymph. In vertebrates with a middle ear) two openings are formed in the skeletal labyrinth, the fenestra rotunda, closed by a membrane, and the fenestra ovale, into which the stapes projects.) The acoustic nerve, which is ganglionated, divides into a vestibular and a cochlear nerve, each with its separate ganglion.)

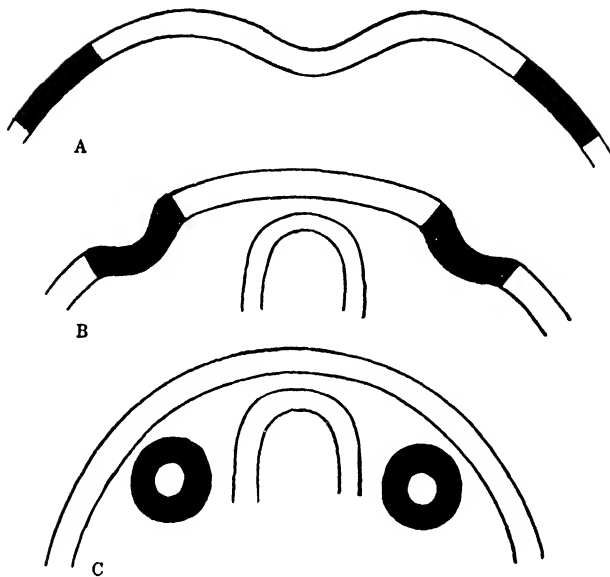


FIG. 179. — Diagrams showing early stages in development of inner ear, A, otic placodes (in black). B, otic pits. C, otic vesicles (otocysts).

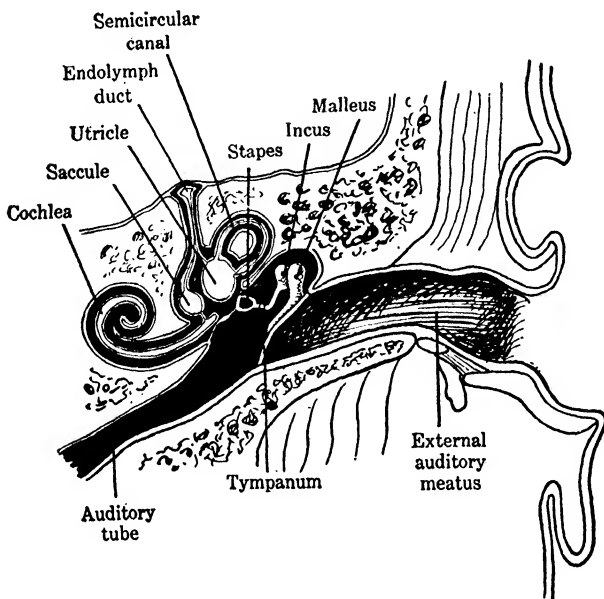


FIG. 180. — Frontal section of human ear. Semi-diagrammatic. (After Howden.)

The middle ear. — The middle ear arises from the first visceral pouch, which constricts into a proximal auditory (Eustachian) tube and a distal tympanic cavity which is separated from the exterior by the tympanic membrane, (a persistent closing plate formed from ectoderm and endoderm. Through the tympanic cavity there is a chain of bones (auditory ossicles) connecting the tympanum with the fenestra ovalis. In anurans and sauropsids, this chain of auditory ossicles consists of the columella and stapes (hyomandibular). In the mammals, the columella is replaced by the incus and malleus, equivalent to two other jaw bones, the quadrate and articular, respectively. The muscles of the middle ear, tensor tympani and stapedial muscles, arise from the mesoderm of the mandibular and hyoid arches, respectively, and are innervated by the facial and glossopharyngeal nerves.

The outer ear. — The external ear consists of the external auditory meatus, derived from the first visceral groove, and the pinna, which arises from tubercles on the mandibular and hyoid arches. It is composed of mesoderm and ectoderm, contains muscles, and is strengthened by cartilage. The innervation is from the facial nerve.

THE FROG (SEE ALSO CHAPTER XI). — In the development of the nose, the nasal groove stage is suppressed. Instead, a thickening develops from the olfactory pit into the mouth as far as the pharynx. This acquires a lumen which connects the olfactory pit to the pharynx. The development of the eye presents no especial peculiarities. The endolymph duct is a dorsal evagination from the otocyst. The semicircular canals are each formed by the appearance of a pair of ridges in the cavity of the utricle which fuse to enclose the cavity of the canal. The saccule gives rise to two ventral diverticula, the cochlea and basilar chamber. The function of the latter is unknown. The tubo-tympanic cavity arises from the first visceral pouch, which in the frog is vestigial and has no cavity. From this rudiment a strand of cells grows dorsad and later acquires a lumen. It loses its connection with the pharynx and moves backward to the ear region where it acquires a secondary connection with the pharynx (Fig. 181). The tympanic membrane is apparently entirely ectodermal. The columella, which connects the tympanum with the inner ear, arises from two primordia: the inner stapedial plate, which is a part

of the otic capsule; and a cartilage derived from the palatoquadrate bar. This cartilage is thought to be homologous with the hyomandibular bone of fishes. The lateral line organs arise from the fragmentation of a placode known as the placode of the tenth cranial nerve, which innervates this series. Similar epibranchial placodes appear on the head and are innervated by the

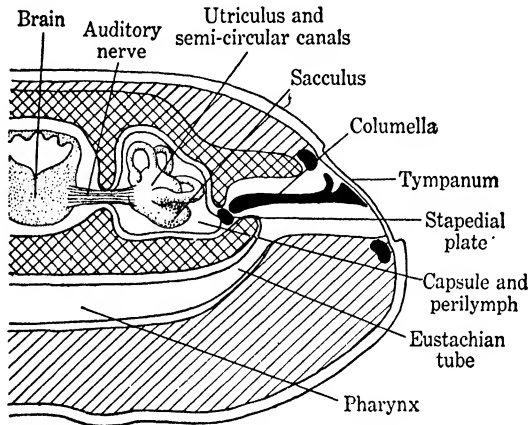


FIG. 181. — *Rana pipiens*, diagram to show the parts of the ear. Schematic cross-section through head.

seventh and ninth nerves. They are larval sense organs and disappear at metamorphosis.

THE CHICK (SEE ALSO CHAPTER XII).— The chick has a cleft palate due to the incomplete fusion of the palatine processes of the maxillae. Jacobson's organ makes a short appearance as a vestigial organ but disappears before hatching. The eye possesses three eyelids, the third (nictitating membrane) arising from a separate fold inside that which forms the upper and lower lids. The pecten is a vascular plate in the vitreous humor, from mesenchyme which enters the choroid fissure. Its function is unknown. The endolymphatic duct arises from the dorsal wall of the otocyst. The semicircular canals arise as out-pocketings of the otocyst prior to its separation into utricle and saccule. The cochlea is more highly developed than in the frog. The tubo-tympanic cavity arises from the first pharyngeal pouch. The tympanum is formed from ectoderm and endoderm and includes a middle layer of mesenchyme. The columella arises from

a stapedial plate and hyomandibular cartilage. The external auditory meatus is short, and no pinna is developed.

MAN (SEE ALSO CHAPTER XIII). — The organ of Jacobson is rudimentary and may completely disappear in the adult. A small fold (*plica semilunaris*) is the representative of the nictitating membrane. The cochlea is highly differentiated. The tube and tympanic cavity form from the first visceral pouch. The tympanum apparently is composed of all three germ layers. There are three auditory ossicles. The stapes is derived from the second visceral arch, while the malleus and incus arise from the first visceral arch. They are thought to represent the quadrate and articular bones of reptiles, respectively. The pinna arises from elevations on the mandibular and hyoid arches.

SUMMARY

The ectoderm gives rise to the epithelial linings of the following structures:

- A. The epidermis, with the
 - apertures of
 - Oral cavity
 - Visceral clefts
 - Cloaca
- B. The neural plate
 - 1. Neural tube
 - Brain and cranial nerves
 - Prosencephalon
 - Telencephalon
 - Diencephalon
 - Mesencephalon
 - Rhombencephalon
 - Metencephalon
 - Myelencephalon
 - Cord and spinal nerves
 - 2. Neural crest
 - Ganglia
 - Cerebrospinal
 - Autonomic
 - Suprarenal gland

C. Sensory placodes

1. Nose
2. Eye (choroid and sclera from mesoderm)
3. Ear (middle ear from endoderm, ossicles from mesoderm)
4. Lateral line organs

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PART IV
ANATOMY OF VERTEBRATE EMBRYOS

CHAPTER XI

THE ANATOMY OF FROG EMBRYOS

In earlier chapters we have discussed the fertilization of the frog's egg (page 57), its cleavage (pages 97, 103), and germ-layer formation (pages 109, 118), and have observed that while the germ layers are being laid down the process is complicated by the early localization of some of the organ systems, notably the sensory-nervous complex (page 129). In this account of later organogeny, three stages of development seem especially significant: first, an early embryo of about 3 mm. body length in which the visceral grooves are apparent, a stage attained in *Rana pipiens* about the second day after the eggs are laid; second, the newly hatched larva of about 6 mm. with external gills developing, about two weeks old; third, a young "tadpole" stage of about 11 mm. with the opercula covering the internal gills, about the age of one month.

These stages are easily identified even though the lengths and ages can be given only approximately, for the rate of development is greatly influenced by the prevailing temperature, and the size of the tadpole is determined largely by external factors, such as the amount of food available.

The student must bear in mind that the sections illustrated in this and the two chapters following are for the sole purpose of giving him starting points from which he is expected to study all the sections in the series furnished him. He will probably never encounter sections exactly like those selected for these illustrations, but he will discover sections very like them from which he can commence his own observations.

A. THE EARLY EMBRYO (3 MM.)

External form. — This stage corresponds approximately to the embryo of $3\frac{1}{2}$ mm. described by Marshall. The head region, through its more rapid growth, has become easily distinguishable from the trunk, which bulges ventrally on account of the large amount of contained yolk, and a well-marked tail bud is present.

The neural folds have fused throughout their length, and enclosed the blastopore. In the head the stomodeum appears as an antero-posterior slit on the anterior ventral surface, and is enclosed by ridges identifiable as the maxillary processes and mandibular arches. On either side and slightly ventral to the stomodeum, are the primordia of the sucker or oral gland. At the dorso-lateral margins the olfactory placodes have begun to evaginate. Lateral bulges on either side of the head are due to the developing optic vesicles. The ear is now in the otic vesicle stage. The gill region shows five visceral grooves. Immediately behind the last arch, a swelling is caused by the developing pronephros. Dorsally, slight furrows indicate the boundaries of thirteen so-

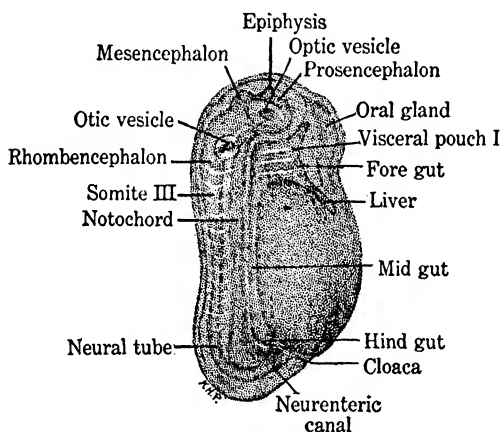


FIG. 182. — 3 mm. frog embryo, viewed from right side as a transparent object. $\times 15$.

mites. Beneath the tail bud, the proctodeum has united with the hind-gut to form the cloacal aperture.

Endodermal derivatives. — The anterior portion of the gastrocoel is now a large fore-gut with a thin-walled lining. From this, on either side, the beginnings of three visceral pouches can be seen. From the fore-gut a nar-

row evagination grows backward into the floor of the mid-gut as the primordium of the liver. The mid-gut is distinguishable by its relatively narrow lumen and thick yolk-laden floor. The small but thin-walled hind-gut opens above into the neurenteric canal by which it is connected with the neurocoel, and opens ventrally to the exterior by way of the proctodeum. An axial rod, the hypochord, is found beneath the notochord. It originates from the roof of the gastrocoel and disappears soon after hatching.

Mesodermal derivatives. — The notochord is large and vacuolated and enclosed by two sheaths. The somites have now attained their maximum number (13) in the trunk, but are not

yet distinguishable in the tail region. The intermediate mesoderm, after a temporary division into nephrotomes, is now re-united into a nephrotomal band in which spaces have appeared opposite the second, third, and fourth somites, indicative of the pronephric tubules which are to develop. A thickening along the

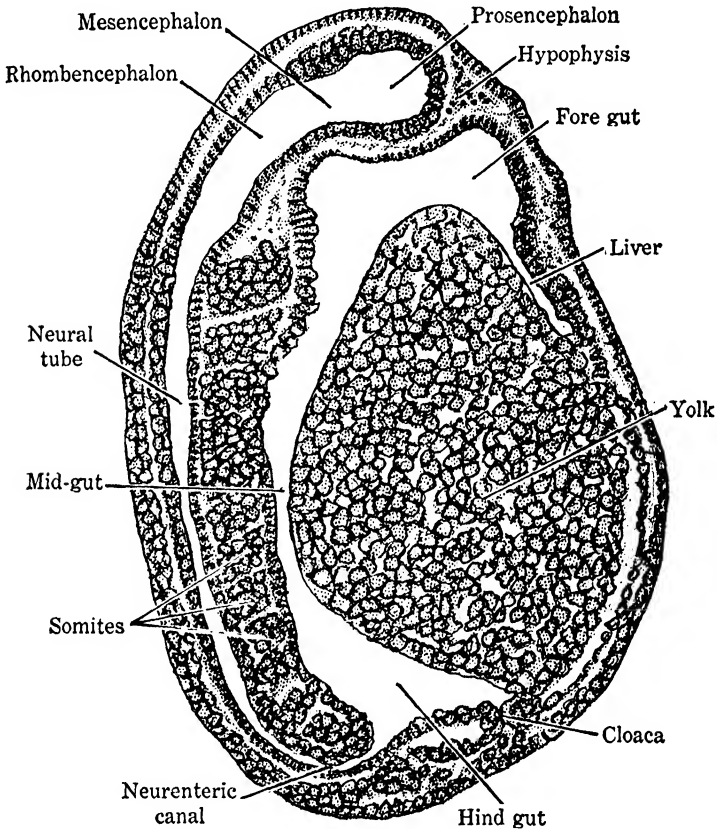


FIG. 183. — 3 mm. frog embryo. Sagittal section. $\times 50$.

nephrotomal band immediately below the ventro-lateral margins of the somites is the primordium of the pronephric duct. Immediately below the floor of the fore-gut, the lateral mesoderm has separated into dorsal splanchnic and ventral somatic layers, while the contained space is the beginning of the pericardial cavity, the only region of the coelom yet apparent.

Ectodermal derivatives. — The epidermis at this stage is ciliated. The neurocoel, as has been remarked above, is con-

nected with the hind-gut by the neurenteric canal. At the anterior end, the brain is distinguishable by its relatively larger lumen and by the cranial flexure over the anterior end of the notochord. The divisions between the three primary vesicles

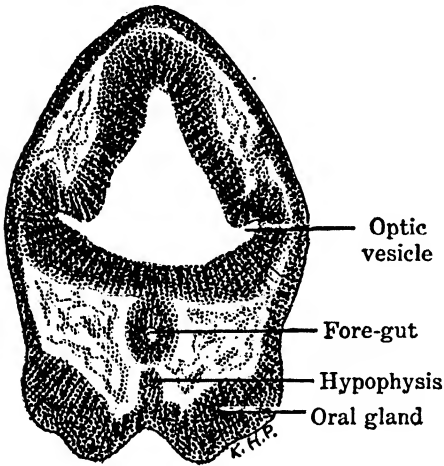


FIG. 184. — 3 mm. frog embryo. Transverse section through optic vesicle. $\times 50$.

are not marked by the constrictions characteristic of many vertebrates, but are distinguished by the following points of reference: the prosencephalon extends to a line projected from a thickening on the floor known as the tuberculum posterius to a point just in front of a similar thickening on the dorsal wall; the mesencephalon, from the boundary of the prosencephalon to a line connecting the tuberculum and a point just behind the

dorsal thickening; the rhombencephalon merges imperceptibly into the spinal cord. From the prosencephalon, the optic vesicles extend on either side and cause the external bulges already noted. From the ventral side of the prosencephalon, a depression, the infundibulum, extends towards the hypophysis, which in the frog grows inward as a solid wedge of ectodermal cells anterior to the stomodeum. Dorsally, the epiphysis appears as a median evagination.

B. THE LARVA AT HATCHING (6 MM.)

External form. — Although the larva, if it may be so called, has emerged from the protecting membranes of egg jelly, the mouth has not yet opened and for several days the yolk is still the sole source of food. The head region is still easily distinguishable from the trunk, while the tail has increased greatly in length and has become bilaterally compressed. In the head, the stomodeal pit has deepened at the anterior end, and the maxillary processes and mandibular arches are more sharply sculptured. The invagination of the nasal (olfactory) placodes has

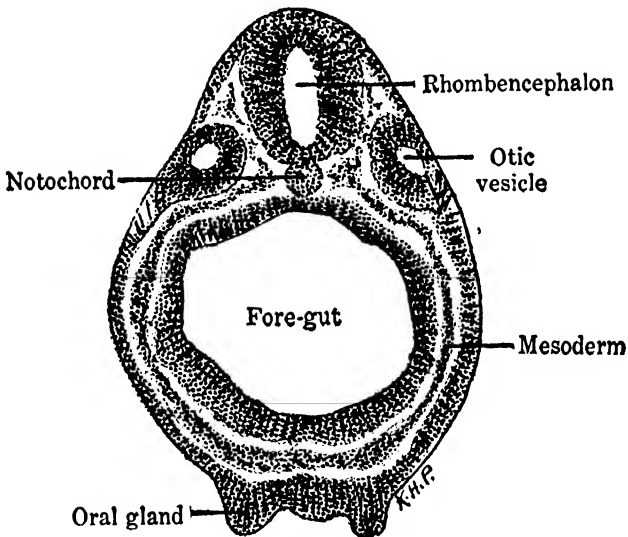


FIG. 185. — 3 mm. frog embryo. Transverse section through otic (auditory) vesicle. $\times 50$.

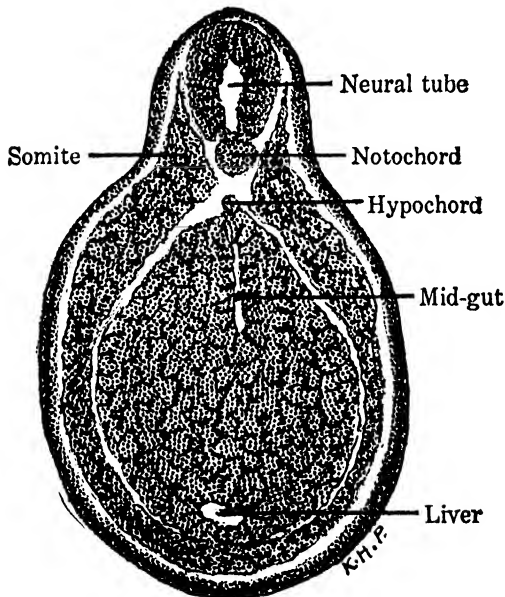


FIG. 186. — 3 mm. frog embryo. Transverse section through mid-gut and liver. $\times 50$.

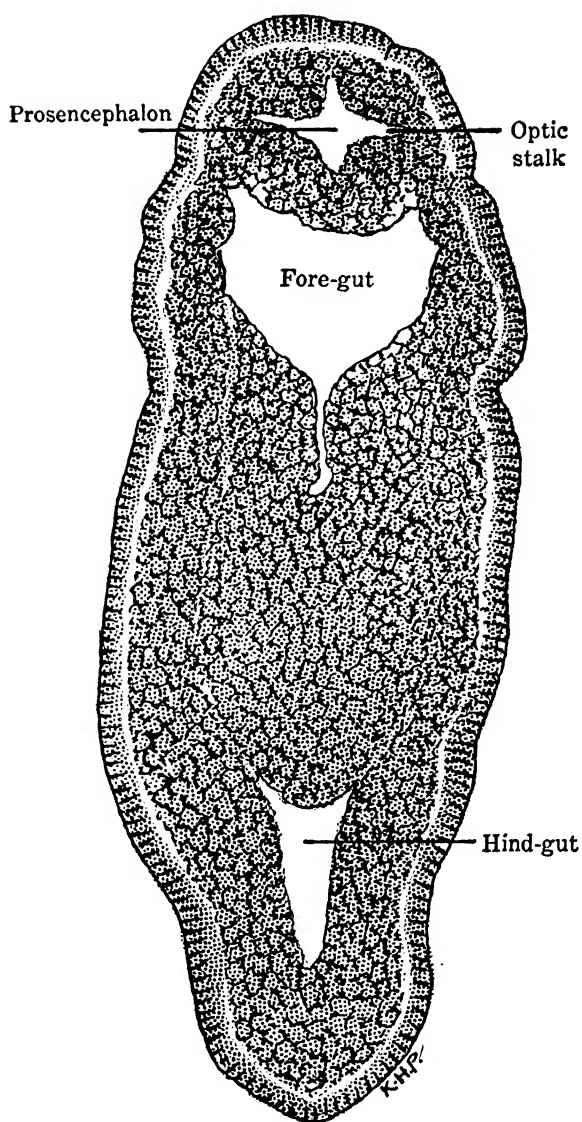


FIG. 187. — 3 mm. frog embryo. Frontal section through optic stalks, liver, and hind-gut. $\times 50$.

continued to the point where they may be called pits, connected to the anterior margins of the stomodeal pit by oro-nasal grooves. The bulge of the eye is still prominent. The primordia of the oral glands have fused to form a U-shaped sucker ventral and posterior to the stomodeum. The visceral grooves are still separated from the visceral pouches by closing membranes, while on the third and fourth arches external gills have appeared. Behind them the pronephric elevation is well marked, and continues backward as a slight ridge marking the pronephric duct. Intersomitic grooves are still apparent. On the ventral side at the base of the tail is the cloacal aperture.

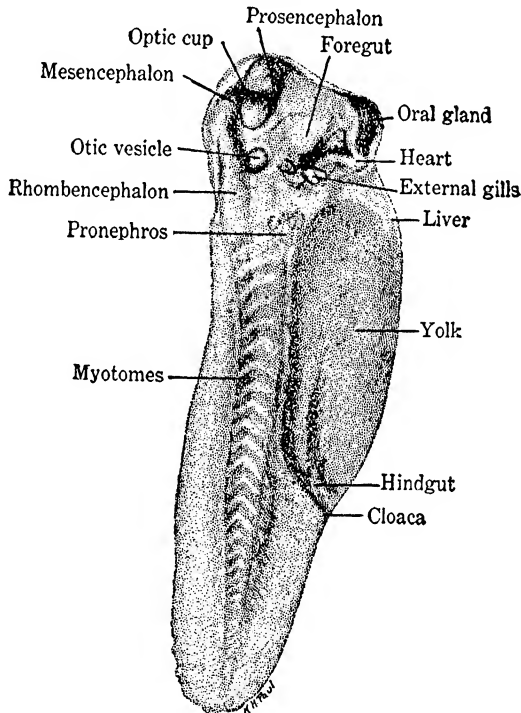


FIG. 188. — 6 mm. frog larva (just hatched). Transparent preparation, viewed from right side. $\times 15$.

Endodermal derivatives. — On either side of the fore-gut are to be seen five visceral pouches, although they would hardly be recognized as such since they are so compressed. A groove on the ventral side of the pharyngeal cavity is the primordium of the thyroid gland. At this stage, also, the dorsal epithelial

bodies of the first two visceral pouches (hyomandibular and first branchial) may be distinguished. The liver diverticulum has increased in length. The hind-gut has lost its connection with the neurocoel through the occlusion of the neurenteric canal, but now receives the posterior ends of the pronephric ducts.

Mesodermal derivatives. — The notochord has grown back into the tail. The somites have now become differentiated into the myotomes, dermatomes, and sclerotomes, while from the myotomes muscle cells have been formed. The pronephros is now established. There are three pronephric tubules, each opening into the coelom by means of a ciliated nephrostome. Opposite these, a mass of capillaries, connected with the dorsal aorta, forms the so-called glomus, equivalent to the separate glomeruli of other vertebrates. The pronephric tubules grow backward into the pronephric ducts, which have acquired lumina. At the time of hatching, the primordia of the heart have fused to form a tube, twisted slightly and almost S-shaped, suspended in the pericardial cavity by a dorsal mesocardium. Two regions may be distinguished, the posterior atrium and anterior ventricle. From the ventricle leads the bulbus, arising from the fusion of paired primordia. This connects with the dorsal aorta, also the result of fusion, by means of aortic arches in the third and fourth visceral arches (vestiges of the first and second aortic arches have already appeared and disappeared). At a slightly later stage, loops from these arches will grow out into the external gills to form a branchial circulation. The anterior ends of the dorsal aortae are prolonged to form the internal carotids, while the posterior ends unite directly above the heart, and just after uniting give off the glomi on either side. Both the somatic and splanchnic venous systems are represented at this stage. Two vitelline veins unite to enter the heart at the sinus venosus. The cardinal veins at this time are represented by irregular lacunar spaces in the head and near the pronephros.

Ectodermal derivatives. — The epidermis is still ciliated. From the prosencephalon the thin-walled cerebral vesicle has appeared. The epiphysis is well marked, and the infundibulum is in contact with the hypophysis. At this time the primordia of cerebrospinal nerves may be distinguished. In the spinal nerves, dorsal roots arise from the ganglia produced by the seg-

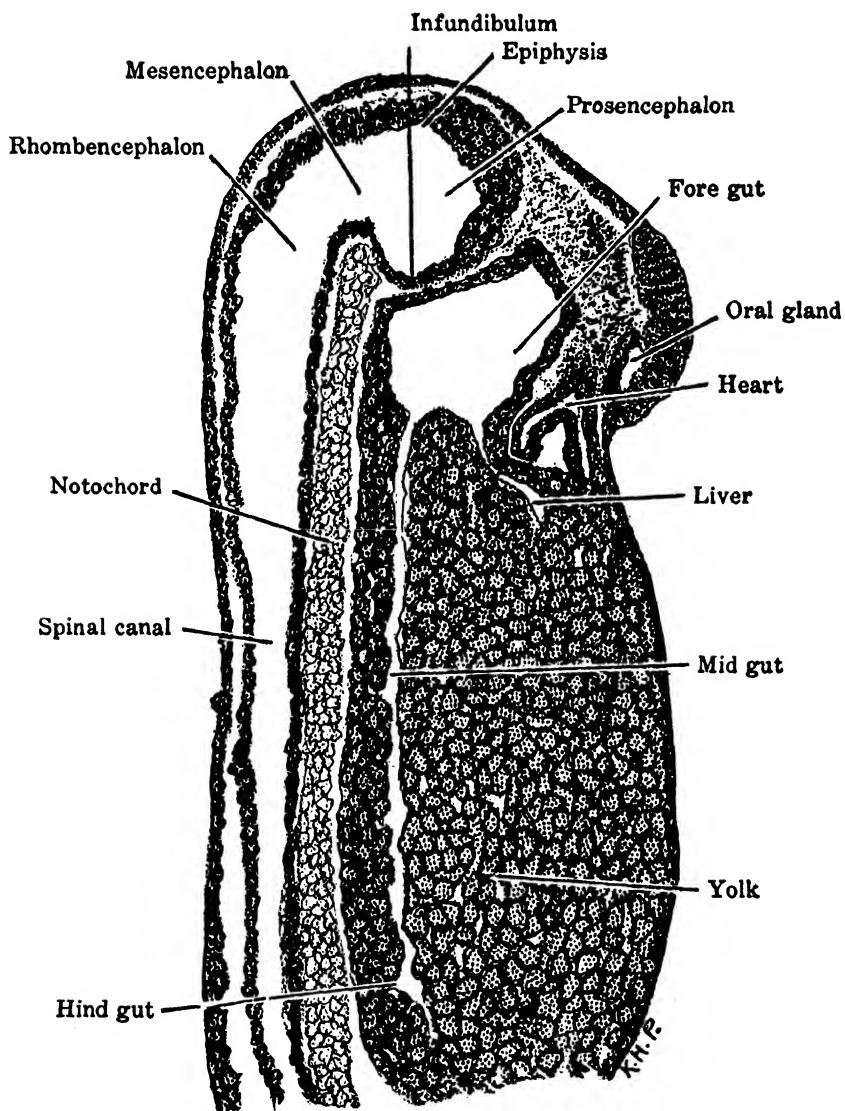


FIG. 189. — 6 mm. frog larva. Sagittal section, anterior portion. $\times 50$.

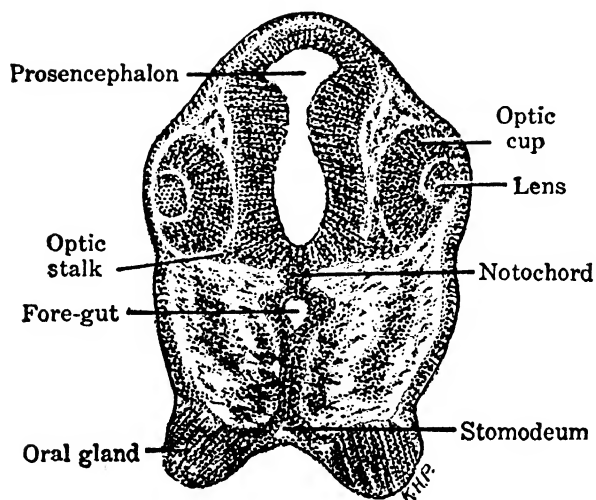


FIG. 190. — 6 mm. frog larva. Transverse section through optic cup. $\times 50$.

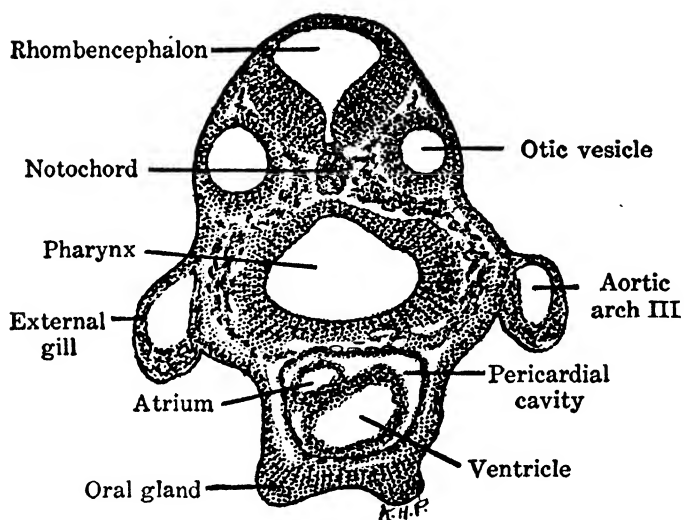


FIG. 191. — 6 mm. frog larva. Transverse section through otic vesicle. $\times 50$.

mentation of the neural crest while the ventral roots arise from neuroblasts in the spinal cord. In the head, four ganglia arise and with each is associated a placode of nervous ectoderm. From the first ganglion and placode, the trigeminal (V) nerve arises. The second combination gives rise to the facial (VII) and acoustic (VIII) cranial nerves, while the remainder of this placode invaginates to form the otic vesicle. The third ganglion and placode produce the glossopharyngeal (IX) cranial nerve, and the

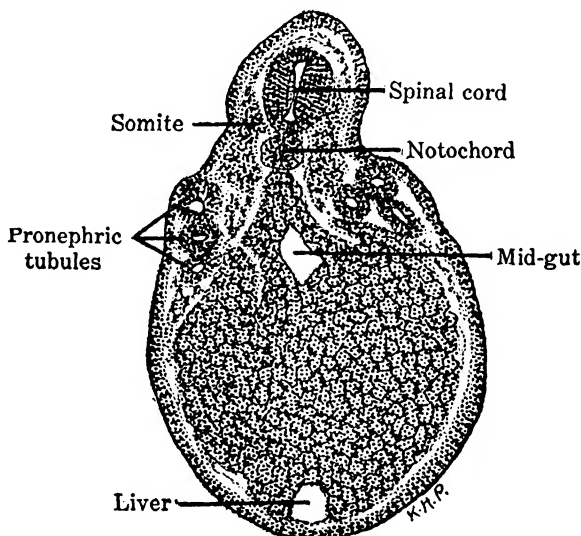


FIG. 192. — 6 mm. frog larva. Transverse section through pronephros. $\times 50$.

fourth gives rise to the vagus (X). The fourth placode grows back as far as the tail, giving off as it goes small groups of cells which later become the lateral line organs of the trunk. Those of the head arise from the second and third placodes. At this time, also, ganglion cells are migrating toward the dorsal aorta to aggregate as the ganglia of the autonomic nervous system. The eye is well advanced in development, as the optic vesicles have invaginated to form the optic cup and the lens placode has separated from the epidermis and acquired a cavity. The ear is in the otic vesicle stage with an endolymphatic duct. The nose is still represented by the nasal pits. From the prolongation of the fourth placode referred to above, the lateral line system is in process of formation.

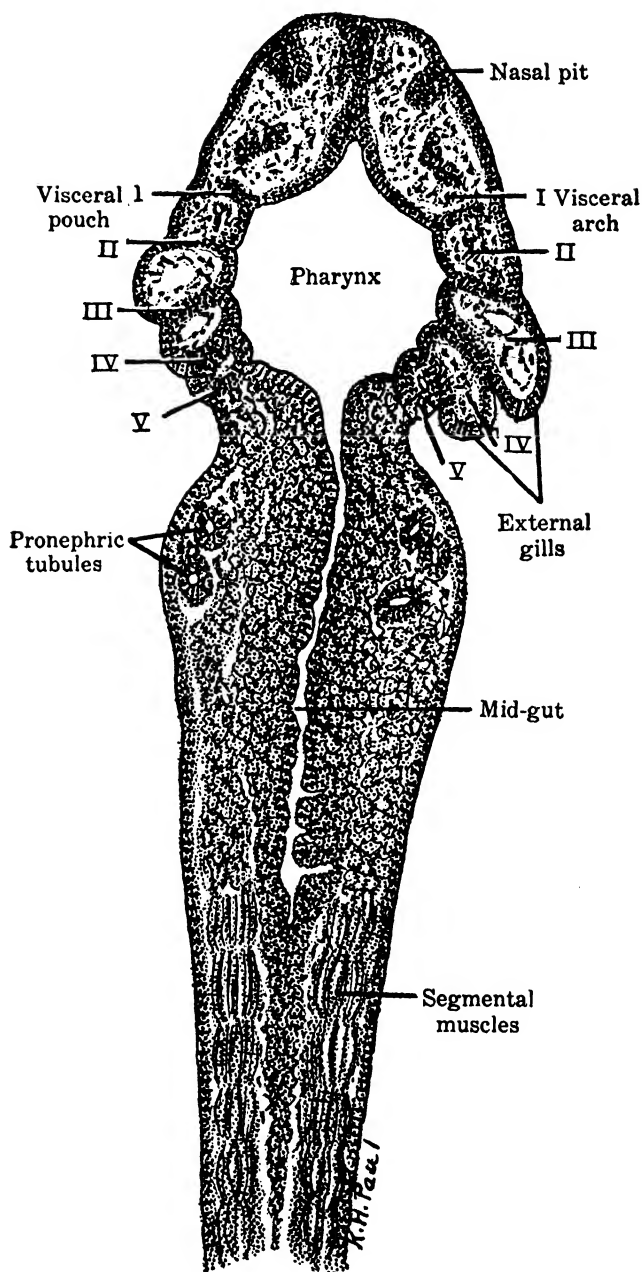


Fig. 193. — 6 mm. frog larva. Frontal section through nasal pit and visceral pouches. $\times 50$.

C. THE YOUNG TADPOLE (11 MM.)

External form. — The head and trunk are now fused into a common ovoid mass, sharply distinguished from the long bilaterally compressed tail. The mouth is open and equipped with horny rasps, while the oral gland is reduced to two vestiges on the ventral side of the head. On the dorsal surface, the large eyes, now functional, protrude slightly. Anterior to these are the external openings of the nasal tubes (external nares). The external gills, which were developing at hatching, have now degenerated and been replaced by internal gills concealed from view by the opercula. On the left side, the opercular aperture serves as a means of egress for the water from which the gills obtain their oxygen. The tail, now two-thirds the length of the tadpole, has a dorsal and a ventral fin. Close to the margin of the latter, at the base of the tail, is the cloacal opening.

Endodermal derivatives. — The mouth has been formed by the breaking through of the oral membrane. From the pharynx, all the visceral pouches except the hyomandibular and the vestigial sixth pouch open to the exterior as visceral clefts; and demibranchs have arisen on the anterior and posterior margins of the third, fourth, and fifth visceral arches and on the anterior margin of the sixth. These are the internal gills which hang down into the opercular cavity. The epithelial bodies from the hyomandibular pouch have disappeared. Those from the second pouch form the thymus gland, while similar buds arise from the third and fourth but presently disappear. The ventral epithelial bodies of the second pouch are said to give rise to the carotid gland, and those of the third and fourth to "parathyroids." The fifth pouch never gains communication with the exterior but gives rise to the ultimobranchial bodies. The thyroid is now separated from the pharynx. In the tadpole the pulmonary organs consist of a pair of thin-walled sacs, the lungs, arising from a laryngeal cavity connected with the pharynx by a narrow opening, the glottis. Posterior to the pharynx comes the esophagus, which was occluded just before the opening of the mouth but now possesses a narrow lumen opening into the stomach, which is not greatly dilated. The vesicle, which formerly represented the liver, persists as the gall bladder and common bile duct, rela-

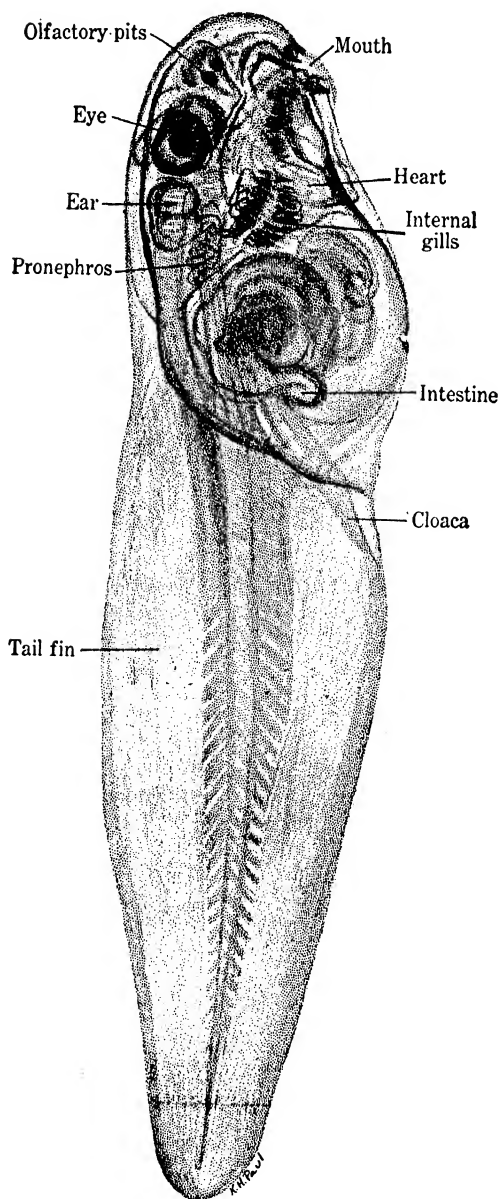


FIG. 194. — 11 mm. frog larva.¹ Transparent preparation viewed from right side.
×15.

¹ Figs. 194–198 inclusive are from preparations loaned me by Dr. A. R. Cahn. In earlier editions they were labelled 9 mm., as measured after preservation.

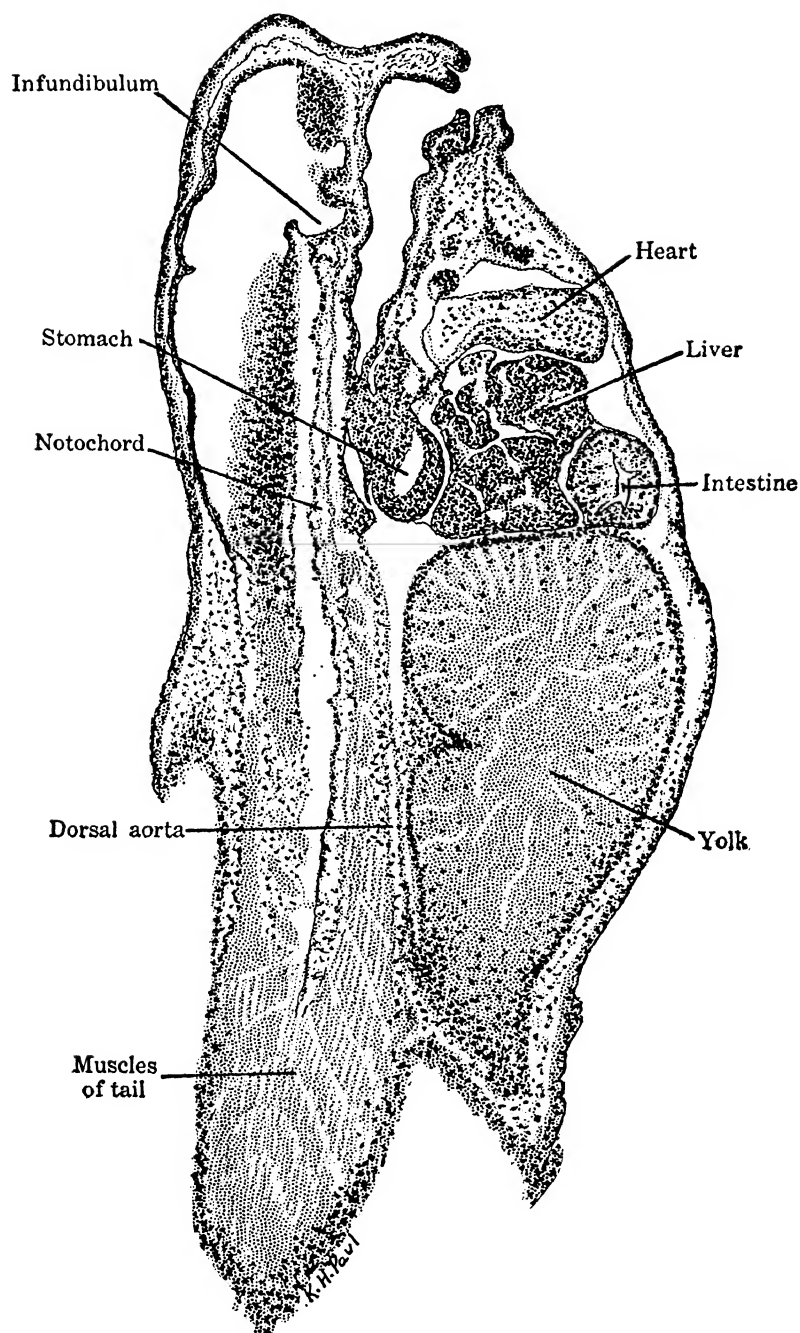


FIG. 195. — 11 mm. frog larva. Sagittal section, anterior part. $\times 40$.

tively small in comparison with the great glandular mass of the liver. Although the pancreas arose from paired primordia of the duodenum, these have now shifted their position so that their ducts open into the common bile duct. The intestine is extremely long and coiled into a double spiral. It terminates in a slightly dilated rectum, opening into the cloacal cavity which also receives the pronephric ducts and opens to the exterior by the cloacal aperture.

Mesodermal derivatives. — The notochord has elongated toward the posterior end, accompanying the growth of the tail. The two most anterior somites have disappeared, leaving eleven in the trunk region and a much larger and variable number in

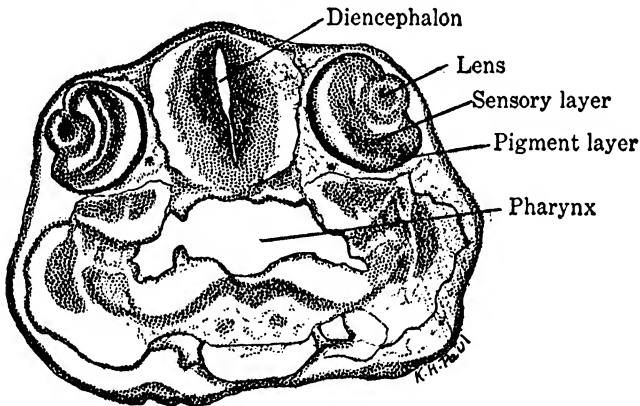


FIG. 196. — 11 mm. frog larva. Transverse section, through eye. $\times 40$.

the tail. In the tail the myotomes have given rise to the dorsal and ventral musculature. The pronephros has become larger and more complicated through the branching of the pronephric tubules. The coelom consists of a pericardial cavity containing the heart, whose myocardia have disappeared, and an abdominal cavity in which the gut is suspended by the dorsal mesentery. These cavities are still continuous up to the time of metamorphosis. In the heart the sinus venosus is now a large transverse sac; the atrium is partially divided by the interatrial septum; the ventricle has thick muscular walls; and the short bulbus opens into the ventral aorta which is divided into proximal and distal portions by a pair of valves. The ventral aorta is divided into

four afferent branchial arteries, the ventral portions of aortic arches III-VI. From these the blood passes through the internal gills by means of capillaries and is conveyed to four efferent branchial arteries, the dorsal portions of the aortic arches referred to above, which in turn lead to the dorsal aortae. The carotid arteries are connected in front of and behind the infundibulum by commissural vessels, and continue forward as the anterior cerebral arteries. From the anterior commissure the basilar run backward and the anterior palatines forward. The pharyngeal

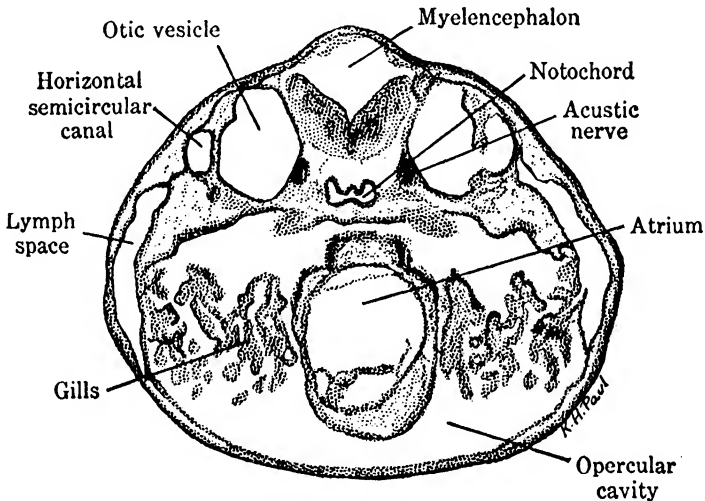


FIG. 197. — 11 mm. frog larva. Transverse section through ear. $\times 40$.

artery, running forward from the dorsal aorta, at a point just posterior to the anterior commissure, represents the dorsal portion of the mandibular arch; the lingual artery arises independently and unites with the first efferent branchial. From the efferent branchial arteries of the sixth arch, the pulmonary arteries grow backward to the lungs. The vitelline veins have been broken up, by their inclusion in the developing liver, into hepatic veins, opening into the sinus venosus, and hepatic-portal veins from the intestine. The anterior cardinal veins are formed by the union of the superior jugular and facial veins and empty into the common cardinals. From the ventral side of the head the inferior jugulars drain into the common cardinals. The posterior somatic veins are the posterior cardinals, which return the blood from the

region of the pronephros into the common cardinals. The lymphatic vessels of the tadpole have arisen from the confluence of numerous, small intercellular spaces in the mesenchyme.

Ectodermal derivatives. — The epidermis is no longer ciliated. The cerebral vesicle is now subdivided into right and left portions, while immediately behind this is the choroid plexus of the diencephalon. The pineal gland is connected with the diencephalon by a small stalk; the pituitary gland has lost all connection with the exterior. In the mesencephalon the optic

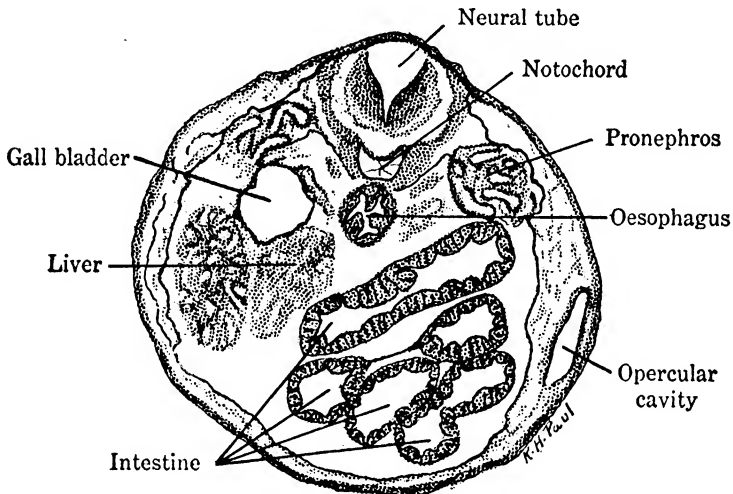


FIG. 198. — 11 mm. frog larva. Transverse section through pronephros. $\times 40$.

lobes are just apparent. The metencephalon is distinguishable by the thickness of its walls as compared with the choroid plexus of the myelencephalon. All cranial nerves and spinal nerves are now established. The eye now contains all elements necessary for functioning; rods and cones of the sensory layer connect with the neurons of the optic nerve; pigment is deposited in the pigment layer; the choroid and sclerotic layers have been formed from mesenchyme; the lens is transparent, as is the cornea formed from the ectoderm. The otocyst is partially divided by a dorsal partition into an outer saccule and inner utricle. The nasal pits have grown backward as solid rods which by now have acquired lumina and will soon open into the pharynx.

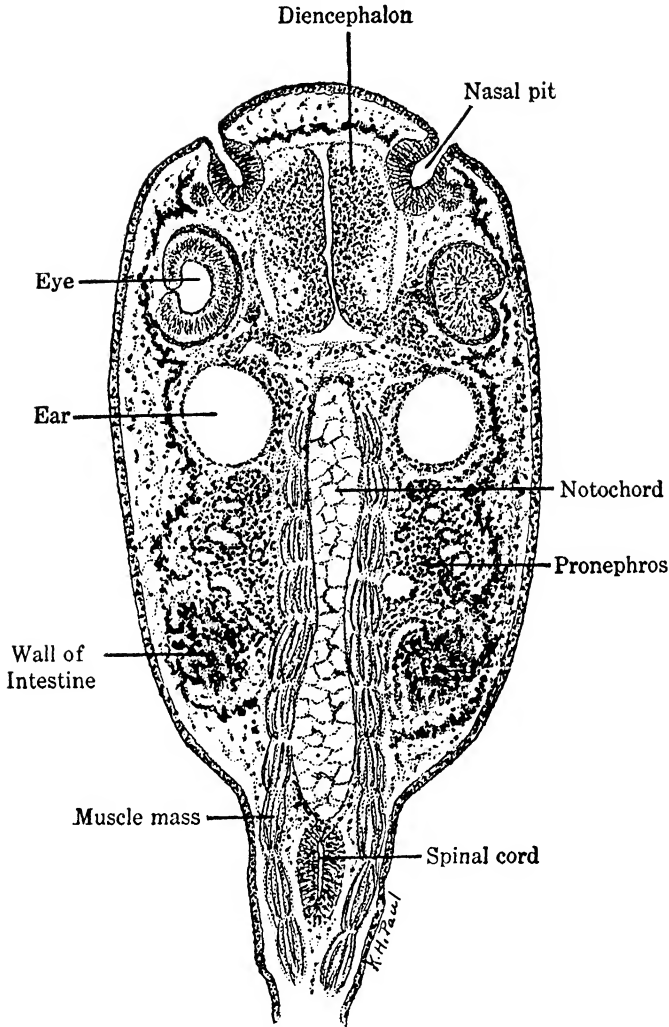


FIG. 199. — 11 mm. frog larva. Frontal section through nose, eye, and ear. $\times 40$.

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CHAPTER XII

THE ANATOMY OF CHICK EMBRYOS

The traditional stages in the development of the chick (*Gallus domesticus*) for laboratory practice are those at the end of each of the first three days of incubation. So many important changes take place during the second day, however, that it is advisable to study an additional stage intermediate between twenty-four and forty-eight hours in age. The chick of thirty-three hours is selected because the form of the embryo is not yet affected by torsion or flexure, and the headfold of the amnion has not yet slipped over the head of the chick.

As it is a well-known fact that, in these first few days of incubation, embryos of the same age have attained varying degrees of development, the length of the embryo has been proposed as a mark of identification. The flexures of the body, however, make this standard impracticable, and the remaining alternative is to select the specific development of some particular structure as a basis of arrangement. For this purpose the number of somites, suggested by Lillie, is admirable. Still, it must be remembered that on account of the effect of temperature upon the rate of development, the number of somites is not correlated exactly with the number of hours of incubation, as may be seen from the following table.

TABLE 12

	Duval	Keibel	Lillie	Patten
About 24 hours	Fig. 76 (24 hrs. 6 S)	Fig. 9, 9A (24 hrs. 7-8 S)	Fig. 59 (25 hrs. 7 S)	Fig. 36 (27 hrs. 8 S)
About 33 hours	Fig. 93 (33 hrs. 16 S)	Fig. 10, 10A (32 hrs. 9 S)	Fig. 63 (33 hrs. 12 S)	Fig. 39 (33 hrs. 12 S)
About 48 hours	Fig. 109 (48 hrs. 27-28 S)	Fig. 16, 16A (52 hrs. 27 S)	Fig. 93 (48 hrs. 27 S)	Fig. 55 (55 hrs. 29 S)
About 72 hours	Fig. 115 (68 hrs. 37 S)	Fig. 18, 18A (67 hrs. 35-37 S)	Fig. 117 (72 hrs. 35 S)	Fig. 63 (72 hrs. 36 S)

A. THE TWENTY-FOUR HOUR STAGE

At the end of the first day of incubation, the chick embryo has completed the period of cleavage (pages 98, 105) and germ-layer formation (pages 111, 121), and is in the early stages of organogeny.

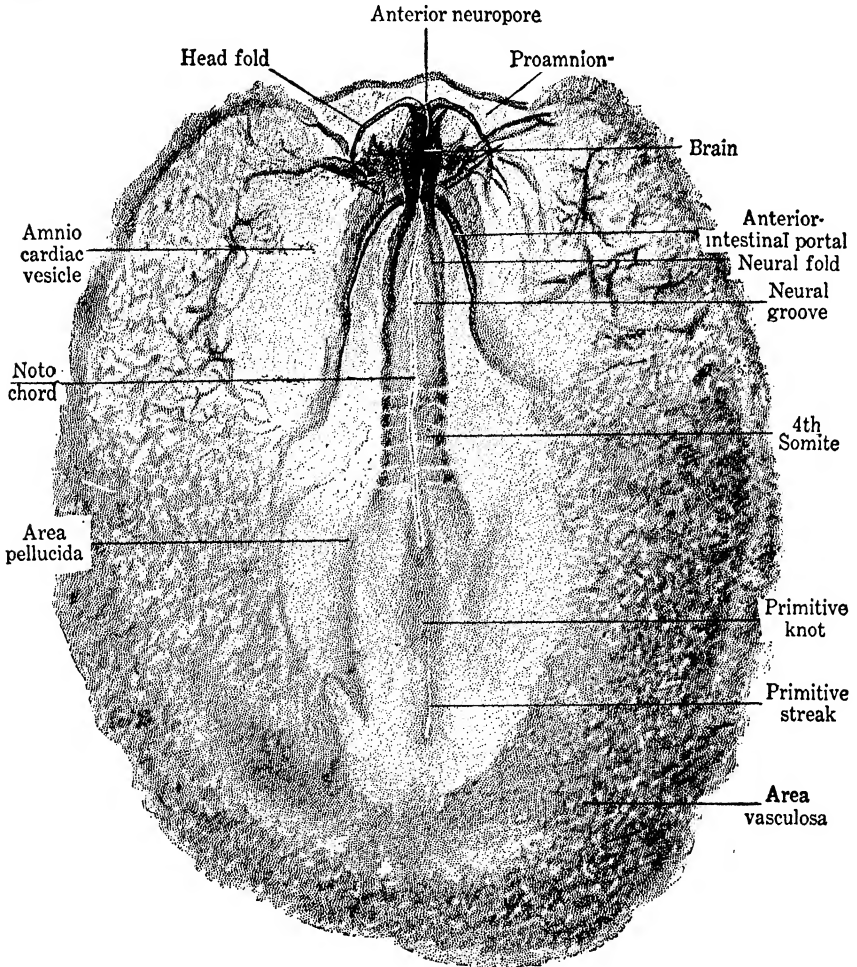


FIG. 200. — 24 hour chick embryo. Cleared preparation from dorsal side. $\times 25$.

External form. — The embryo, 3.3 mm. in length, lies along the axial line of the slipper-shaped area pellucida which in turn is surrounded by the crescent-shaped area vasculosa, whose anterior horns, separated by the proamnion, reach about to the level of

tip of the head. At the anterior end, the head fold of the embryo is lifted above the proamnion from which it is separated by the subcephalic pocket. In the head fold is contained the fore-gut,

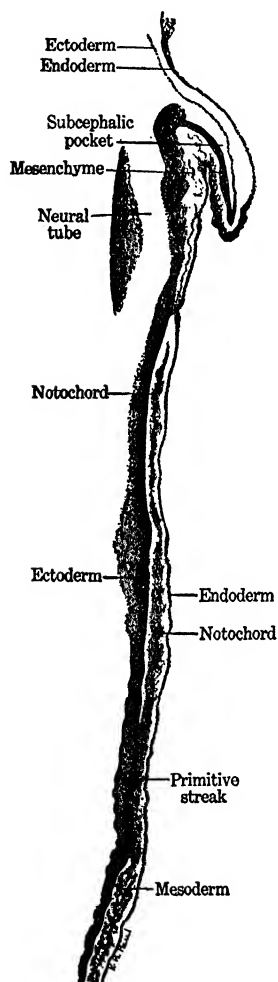


FIG. 201. — 24 hour chick embryo. Sagittal section. $\times 37\frac{1}{2}$.

0.59 mm. in length, which opens at its posterior end into the yolk cavity by means of the anterior intestinal portal. On either margin of the portal the primordia of the vitelline veins are to be recognized in thick bands of splanchnic mesoderm. The neural plate has already given rise to the neural folds which extend back as far as the first somite. They have united just posterior to the region where the optic vesicles are to appear and thus have given rise to a neural tube 0.3 mm. in length, which is widely open in front and behind as the anterior and posterior neuropores, respectively. Behind the head fold the axial mesoderm is segmented into six somites. Between the neural folds the notochord can be recognized as a faint line which joins, at its posterior end, the primitive streak, now reduced to 0.83 mm. in length.

Endodermal derivatives. — The only differentiation which has taken place in the endoderm consists of the establishment of the fore-gut by means of the folding off of the head from the proamnion. As this process continues the fore-gut will be lengthened at the expense of the widely open mid-gut, and the anterior intestinal portal will progress steadily backward.

Mesodermal derivatives. — The mesoderm proper does not extend into the head, but a loose aggregate of mesenchyme derived from it is present. Posterior to the head the axial mesoderm is divided into six somites. Transverse sections show that

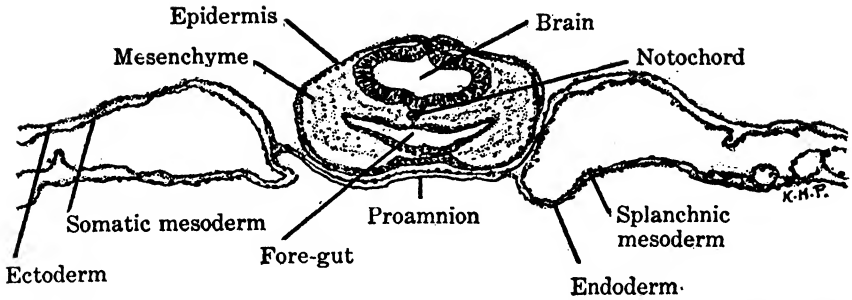


FIG. 202. — 24 hour chick embryo. Transverse section through brain region. The neural folds have met but are not yet fused together. $\times 50$.

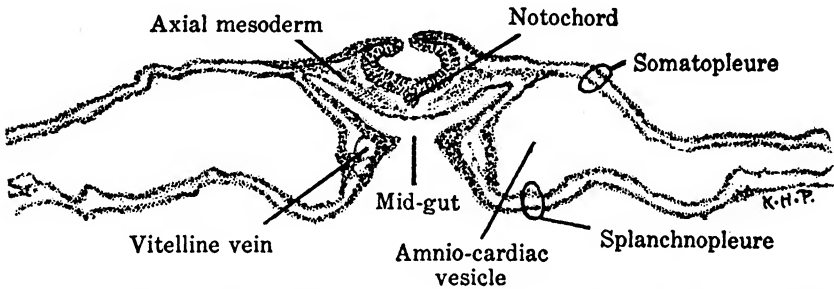


FIG. 203. — 24 hour chick embryo. Transverse section through region of intestinal portal. $\times 50$.

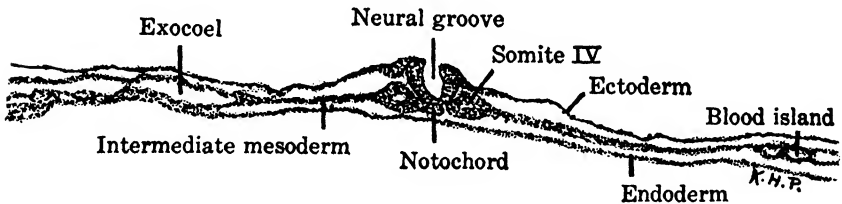


FIG. 204. — 24 hour chick embryo. Transverse section through fourth somite. $\times 50$.

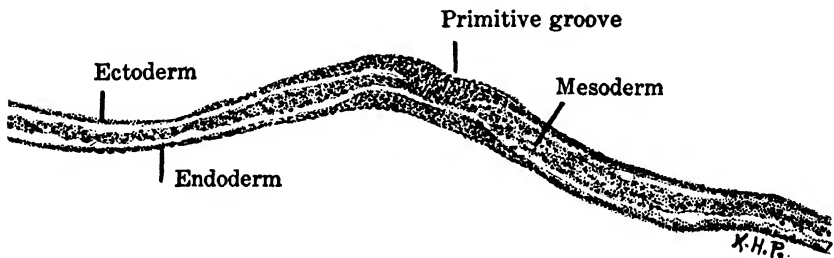


FIG. 205. — 24 hour chick embryo. Transverse section through primitive streak. $\times 50$.

each has a minute cavity, or myocoel. The intermediate mesoderm does not divide into nephrotomes as in the frog. The lateral mesoderm is divided into the somatic and splanchnic layers. In the latter, numerous blood islands appear and give the characteristic mottled appearance to the area vasculosa. The coelom of the embryo is continuous with that of the extra-embryonic regions, or exocoel. In the region on either side of the head, between the proamnion and the intestinal portal, the coelom is distended into an amniocardiac vesicle, so called because the somatopleure will contribute to the head fold of the amnion, while the splanchnic mesoderm will give rise to the primordia of the heart, and the cavities of the vesicles will unite to form the pericardial cavity. The notochord, from its point of origin, the primitive streak, extends forward into the head.

Ectodermal derivatives. — The ectoderm at this stage consists of the elongate neural plate, with its groove and folds which are already in process of fusion, and the epidermis or non-nervous ectoderm.

B. THE THIRTY-THREE HOUR STAGE

External form. — In the chick embryo, after thirty-three hours' incubation, the length has increased to 4.3 mm. There is a slight bending of the head downward over the end of the notochord, foreshadowing the cranial flexure. The area vasculosa, in which the blood islands are being converted into capillaries, now has grown in toward the embryo, so that the area pellucida persists only around the head and tail regions. The anterior horns of the area vasculosa have met in front, completely enclosing the proamnion. The head has increased in length not only by actual forward growth but also by the backward extension of the lateral margins of the head fold, so that the enclosed fore-gut is now 1 mm. long. The vitelline veins are prominent at the margins of the intestinal portal and continue on the ventral side of the fore-gut to meet at the posterior end of the heart, which is now a single tube, slightly bent toward the right. The neural folds are fused as far back as the eleventh somite, where the posterior neuropore is now known as the rhomboidal sinus. The anterior neuropore is about to close, and in the head the neural tube shows three regions of dilation which represent the

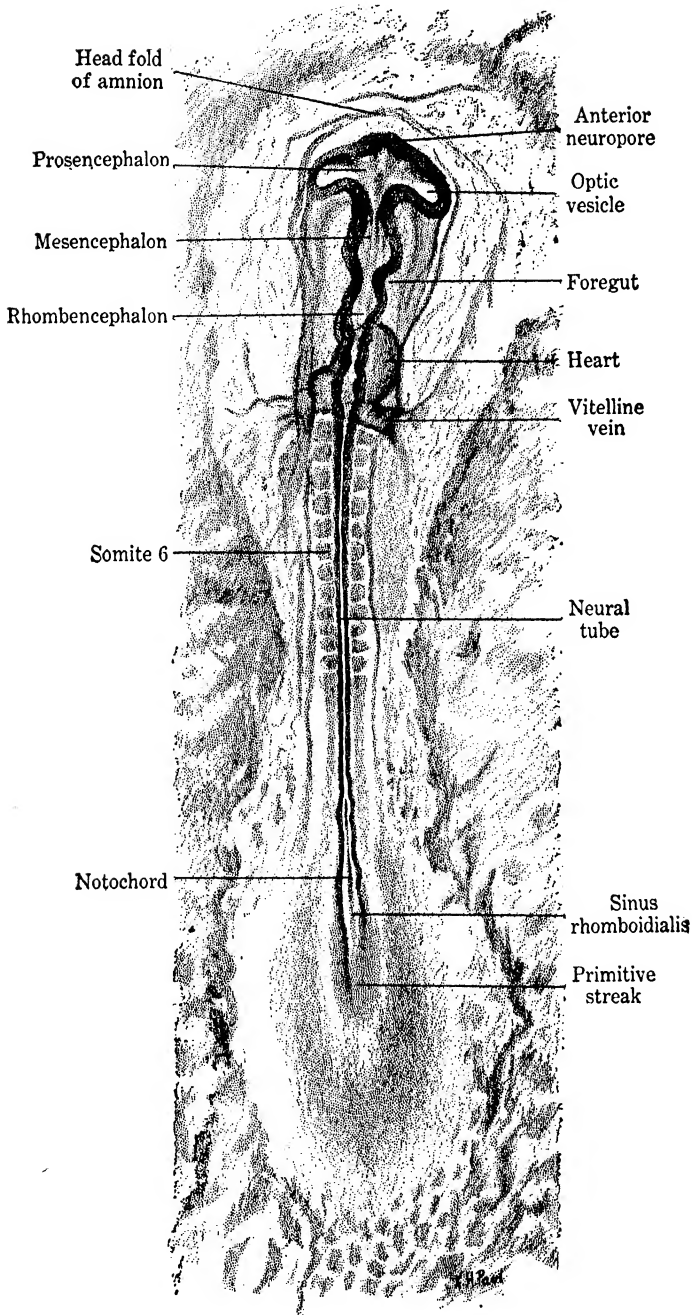


FIG. 206. — 33 hour chick embryo. Cleared preparation from dorsal view. $\times 25$.

fore-brain, mid-brain, and hind-brain, respectively. The sides of the fore-brain are evaginating to produce the optic vesicles.

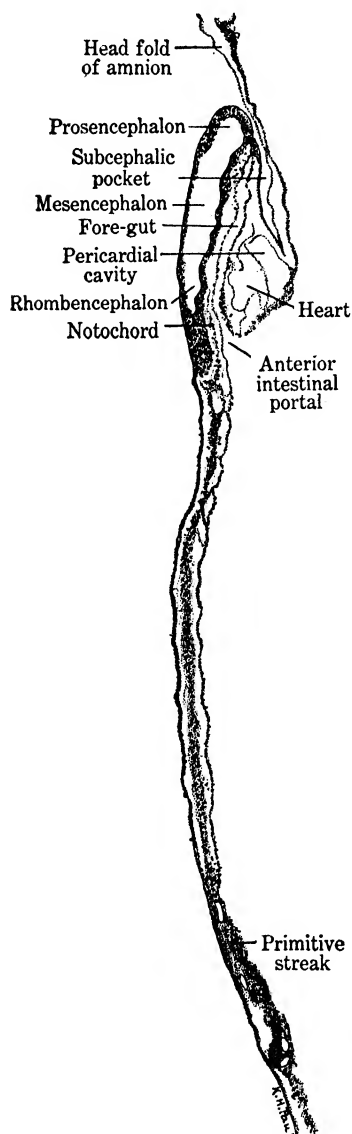


FIG. 207. — 33 hour chick embryo. Sagittal section. $\times 25$.

In the hind-brain, five neuromeres can be identified. Twelve somites may be counted. The notochord extends forward to the fore-brain from the primitive streak which is now reduced to 0.3 mm.

Endodermal derivatives. — The anterior end of the fore-gut is in contact ventrally with the stomodaeum separated only by the oral plate, composed of ectoderm and endoderm. At the sides, the walls of the fore-gut are fused to the ectoderm at points where the first visceral pouches (hyomandibular) will be located.

Mesodermal derivatives. — The somites now number twelve, and myocoels are still apparent. The mesomere is still unsegmented, but pronephric tubules have appeared in the region corresponding to somites 5-12. The four posterior tubules are growing back to form the pronephric duct. In the splanchnic mesoderm the blood islands are being converted into capillaries. The vitelline veins are prominent and continue forward into the heart, of which the endocardium and myocardium are distinct. The heart is supported by the dorsal mesocardium, the ventral mesocardium having disappeared.

The primordial tubes, from the fusion of which the heart arose, continue forward as the ventral aortae which bend around the pharynx (first aortic arches) and

continue backward along the dorsal surface of the pharynx as the dorsal aortae. At the level of the primitive streak they are lost in a capillary nexus which foreshadows the vitelline arteries. From a point immediately in front of the optic vesicle, the anterior cardinals course backward on either side of the neural tube, bending down ventrally to enter the heart with the vitelline veins. The notochord is slightly bent at the anterior end.

Ectodermal derivatives.—The neural folds now extend to the eleventh somite and have fused throughout the length of the head. The anterior neuropore is almost closed. The three

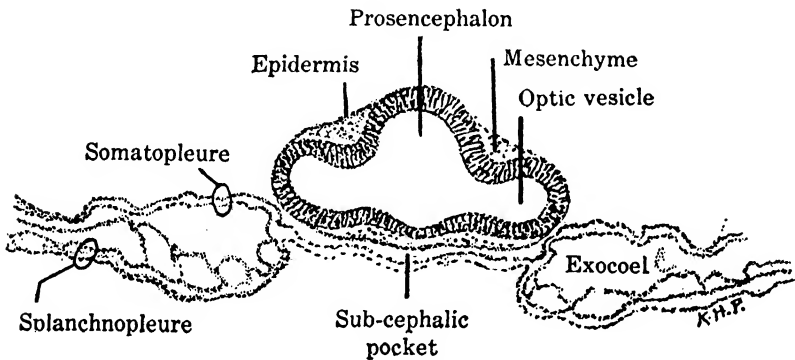


FIG. 208. — 33 hour chick embryo. Transverse section through optic vesicles. $\times 50$.

dilations which represent the prosencephalon, mesencephalon, and rhombencephalon are distinct. From the prosencephalon the two optic vesicles extend to the ectoderm of the sides of the head. Five neuromeres may be identified in the rhombencephalon. It has been asserted that in earlier stages three neuromeres may be identified in the prosencephalon and two in the mesencephalon, while the first of the five noted above has resulted from the fusion of two original neuromeres destined to give rise to the metencephalon. At about this time a shallow depression in the floor of the prosencephalon, just in front of the tip of the notochord, marks the appearance of the infundibulum. The auditory placodes may sometimes be seen in sections as thickenings at the level of the constriction separating the last two neuromeres on either side.

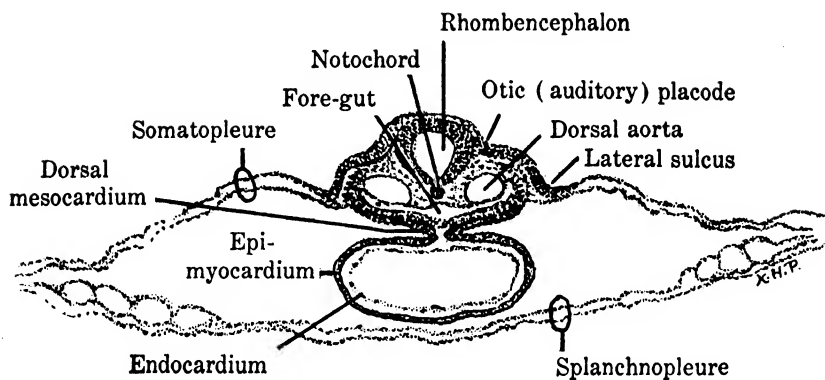


FIG. 209. — 33 hour chick embryo. Transverse section through otic placodes. $\times 50$.

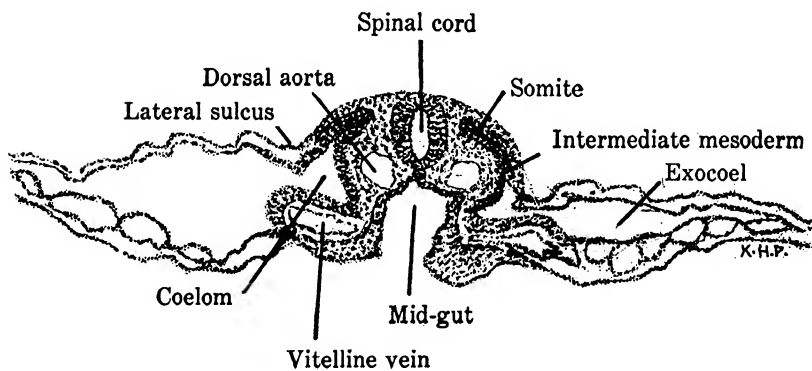


FIG. 210. — 33 hour chick embryo. Transverse section through vitelline veins. $\times 50$.

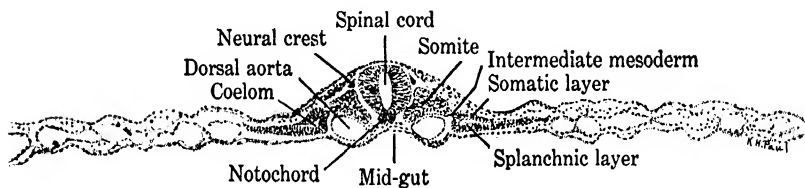


FIG. 211. — 33 hour chick embryo. Transverse section through sixth somite. $\times 50$.

C. THE FORTY-EIGHT HOUR STAGE

External form. — The chick at the end of the second day of incubation has usually attained a length of 7 mm., but the form of the body has been altered profoundly. As the head has been lifted away from the blastoderm, it has increased greatly in size,

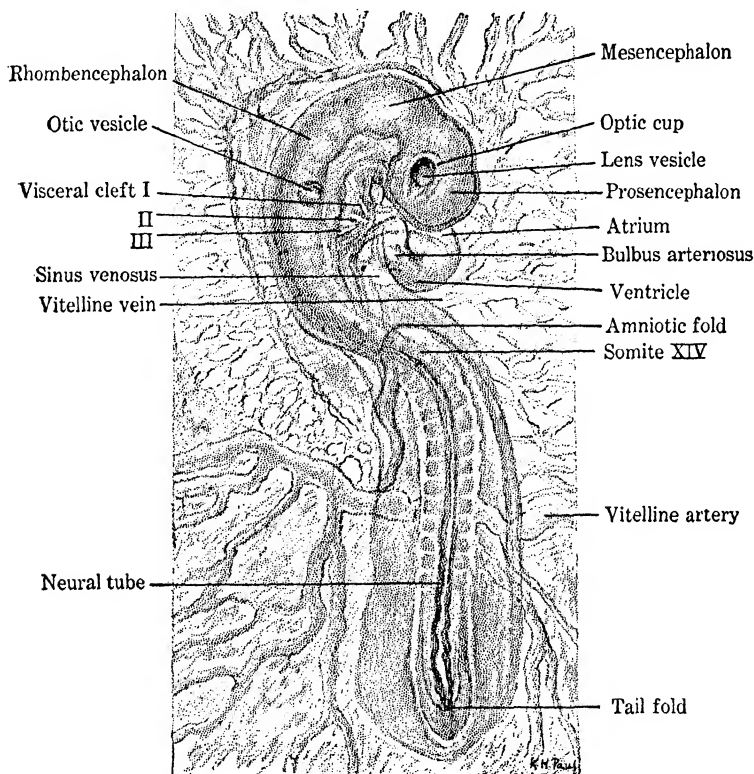


FIG. 212. — 48 hour chick embryo. Transparent preparation from dorsal view (head from right side). $\times 15$.

and the cranial flexure, which was just appearing in the thirty-three hour chick, has become so pronounced that the anterior end of the head is directed backwards. With this growth and flexure the head is twisted normally to the right, until it lies on one side, a phenomenon known as torsion. At forty-eight hours, this torsion involves the chick as far back as the seventeenth somite. The posterior end of the chick lies in its original position, and at the extreme caudal end a tail fold is being formed. In the

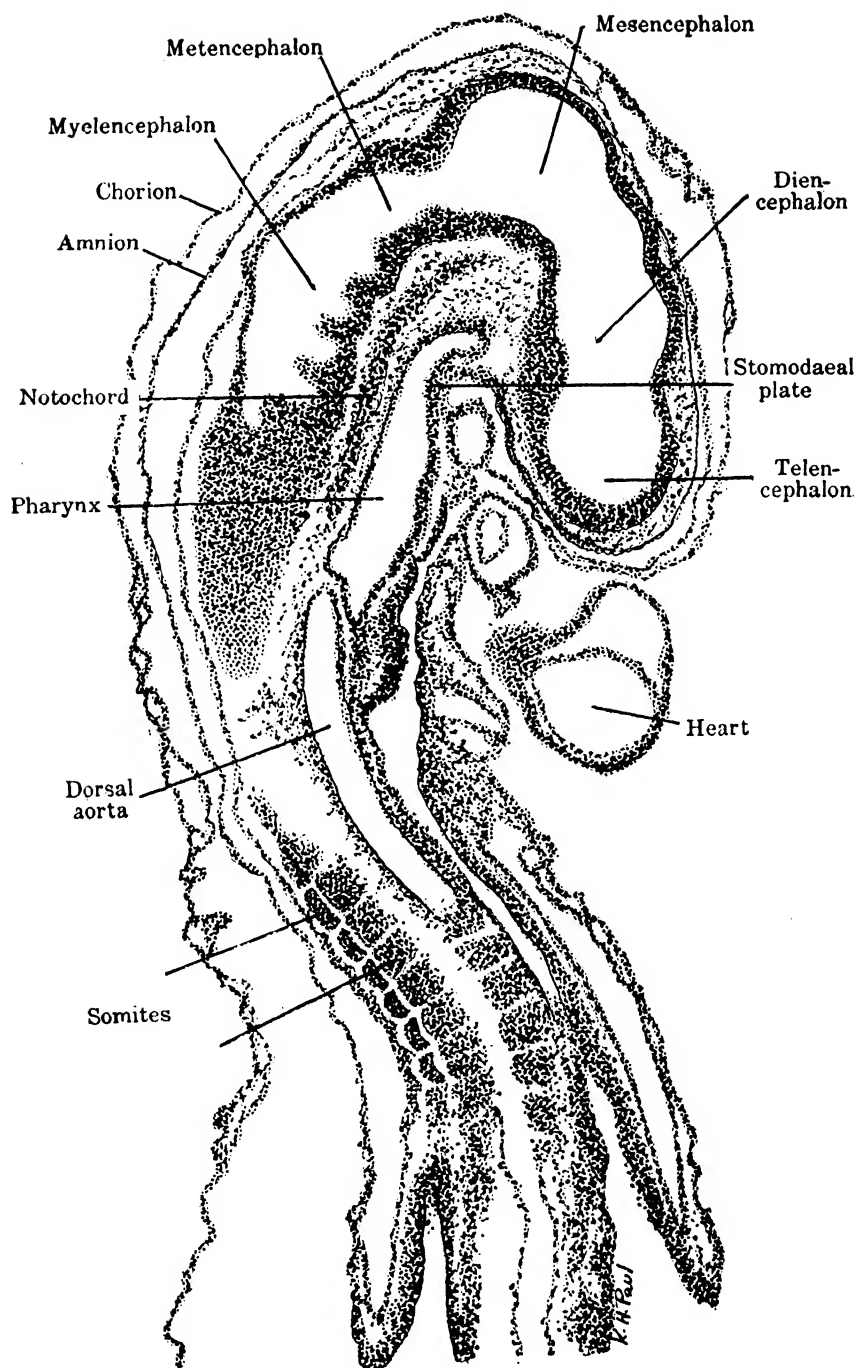


FIG. 213. — 48 hour chick embryo. Head in sagittal section, somite region in frontal section due to torsion. $\times 50$.

area vasculosa the capillaries have formed attachments with the vitelline arteries and veins, and at the border of this area is a circular vessel, the sinus terminalis. The fore-gut is now 1.4 mm. in length, and the first of the three visceral pouches now communicates to the exterior following the rupture of the closing plate which separated it from the corresponding visceral groove. The second and third visceral grooves are apparent, but their closing plates are still unperforated. In the visceral arches the first three aortic arches are apparent, arising from the ventral aorta. The heart is now twisted so that the ventricular loop is upper-

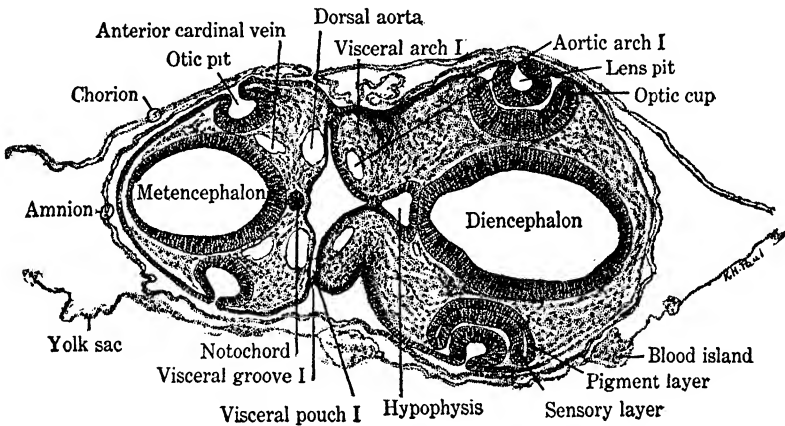


FIG. 214. — 48 hour chick embryo. Transverse section through otic pit and optic cup. $\times 50$.

most. The vitelline veins are large and conspicuous, as are the vitelline arteries which leave the body at the level of the twenty-second somites. The neural tube is completely closed. In the head the five definitive regions of the brain are outlined, the prosencephalon having given rise to the telencephalon and diencephalon, and the rhombencephalon to the metencephalon and myelencephalon. The eye is now in the optic cup stage, and the invagination of the optic vesicle continues down the stalk to form the choroid fissure. The lens is in the form of a pit which has almost attained the vesicle stage. The ear is represented by an otic pit which, owing to the cervical flexure, is about on a level with the eye. There are twenty-seven somites at this stage. The primitive streak is found only in the tail fold. At this time

the head fold of the amnion has grown back over the chick as far as the sixteenth somite.

Endodermal derivatives. — The stomodeum, an ectodermal invagination from the ventral surface of the head fold, has formed the oral membrane by contact with the fore-gut a little back of its most anterior point. Hence there is a blind pocket in front of the oral plate, known as the preoral gut. Three visceral pouches are present, the first of which opens into the corresponding visceral furrow following the rupture of its closing membrane. The primordium of the thyroid is represented by a ventral depression in the floor of the pharynx at the level of the second visceral pouches. The primordia of the lungs (sometimes difficult to distinguish) extend to the level of the sinus venosus. The liver arises at the level of the anterior intestinal portal from two evaginations of the endoderm, one below and one above the meatus venosus. The mid-gut now has two shifting boundaries, the anterior intestinal portal and the posterior intestinal portal. The latter is barely apparent as the opening of a shallow endodermal pocket or hind-gut in the tail fold.

Mesodermal derivatives. — The somites, twenty-seven in number, show a varying degree of specialization, with the most advanced at the anterior end. In these two regions can be distinguished: a loose aggregate of cells at the median ventral angle (the sclerotome); and a cap of epithelial cells at the lateral dorsal angle. The cells of this cap nearest the epidermis will form the dermatome, while those nearest the neural tube will form the myotome.

The pronephric tubules in the more anterior somites have disappeared and mesonephric tubules are appearing in the mesomere posterior to the thirteenth somite. The pronephric (now the mesonephric) duct has acquired a lumen but has not yet attained its complete backward growth.

The heart is still tubular, but the ventricular limb of the cardiac loop has grown back and over the atrial limb so that the ventricular region is now caudal and dorsal with relation to the atrial region. Three aortic arches are present as a rule, but infrequently the third has not developed. From the first aortic arch a network of capillaries extends into the head. From these the carotid arteries will be formed. The dorsal aortae have fused

from a point back of the sixth somite as far as the level of the fifteenth somite. The vitelline arteries leave the dorsal aortae at the level of the twenty-second somite but the aortae continue

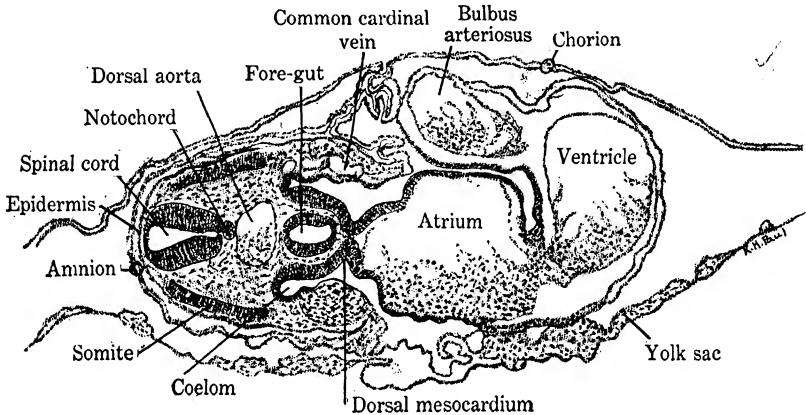


FIG. 215. — 48 hour chick embryo. Transverse section through heart. $\times 50$.

backward as the caudal arteries to the last somite. The vitelline veins are fused at their point of entrance into the heart as the sinus venosus. The anterior cardinals are prominent and extend from a capillary plexus in the head back toward the heart, where they

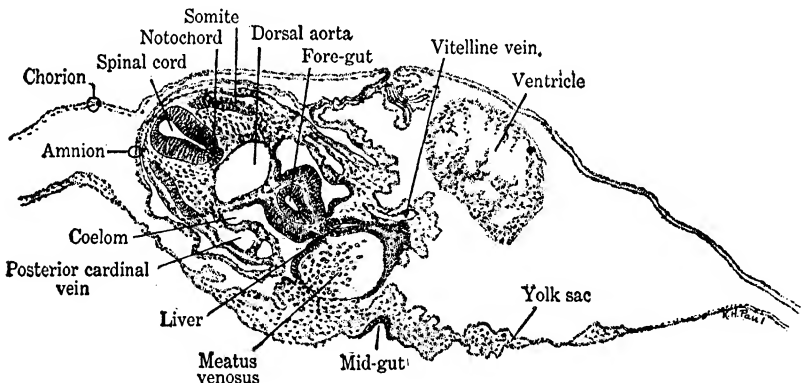


FIG. 216. — 48 hour chick embryo. Transverse section through liver. $\times 50$.

are joined by the posterior cardinals and proceed as the common cardinals to enter the heart in the angles between the sinus venosus and the vitelline veins. The posterior cardinals may be traced back to the last somite. The heart of the chick commenced

beating at the forty-fourth hour of incubation, so that the course of the blood is through the ventral aorta to the aortic arches and thence to the dorsal aorta. From the first aortic arch a network of capillaries supplies the head with blood (which is returned by way of the anterior cardinals). The main current of the stream passes down the dorsal aortae to the point where these fuse to form the median dorsal aorta. From the dorsal aorta, the somites are supplied by capillaries, which will later become the intersegmental arteries. This blood is returned through the posterior cardinals. Leaving the dorsal aorta by way of the vitelline arteries, the blood passes through the capillaries of the area vasculosa to the sinus terminalis, and thence to the capillary drainage of the vitelline veins which return it to the heart.

The notochord is bent, not only at its tip (cranial flexure) but also at the point where the myelencephalon merges with the spinal cord (cervical flexure).

Ectodermal derivatives.—The brain now has acquired its five definitive vesicles. The telencephalon is enlarged but shows

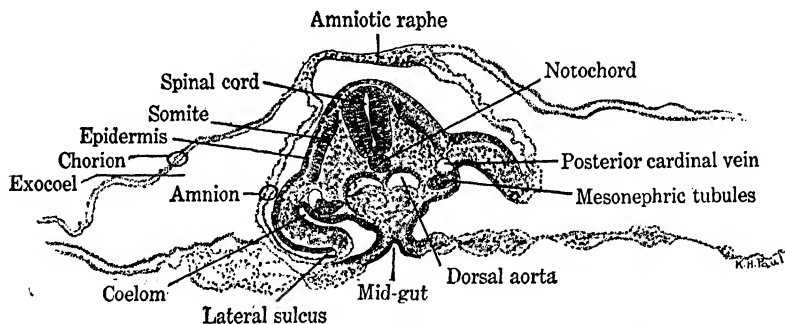


FIG. 217. — 48 hour chick embryo. Transverse section through mesonephros. $\times 50$.

no particular differentiation. From the diencephalon project the constricted optic stalks which bear the optic cups with their inner sensory layer and outer pigmented layer. (The pigment will not arise until later.) The invagination by which the cups were formed continues down the stalk as the choroid groove. On the ventral surface of the diencephalon the infundibulum has deepened. Growing in toward it from the stomodeum is an ectodermal invagination, the hypophysis, which will fuse with the infundibulum to form the pituitary gland. The lens of the eye

is in the pit stage, resulting from the invagination of a sensory placode. When the process is complete, the lens will be a vesicle completely withdrawn beneath the surface of the ectoderm, as will the otic vesicle, the primordium of the inner ear. Along the rhombencephalon and cord, the neural crest is to be seen as a narrow band of cells on each dorso-lateral angle.

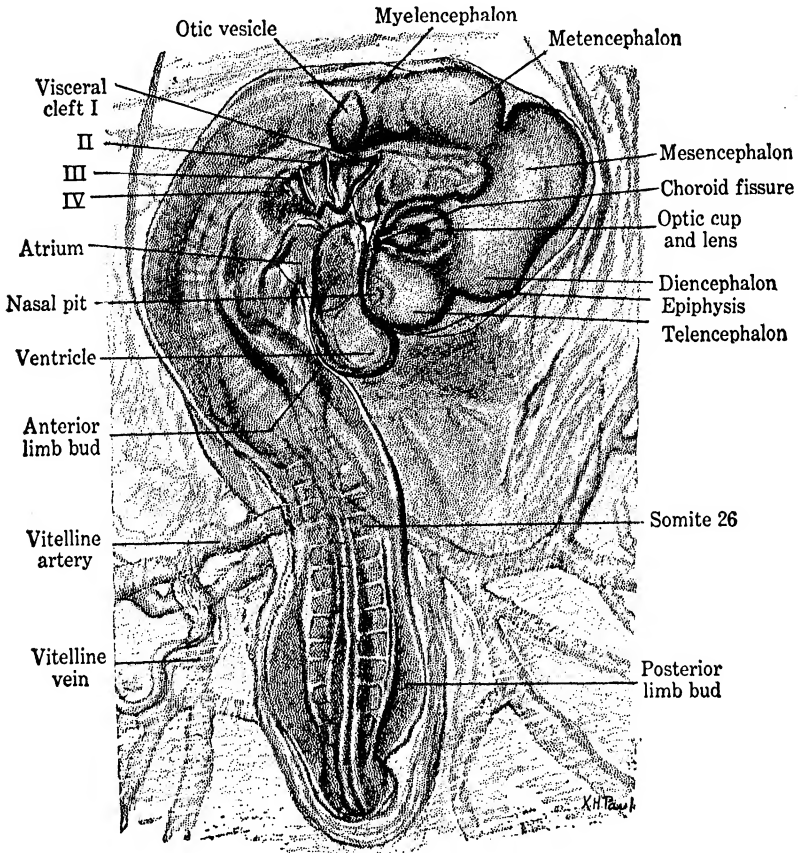


FIG. 218. — 72 hour chick embryo. Transparent preparation from dorsal view, head seen from right side. $\times 15$.

D. THE SEVENTY-TWO HOUR STAGE

External form. — At the end of the third day of incubation, the total length of the embryo is 9.5 mm., but the curvature of the body is so great, on account of the cranial and cervical flexures in addition to the newly developed caudal flexure, that the greatest length, from neck to tail, is 7 mm. Torsion involves the

body as far back as the vitelline arteries and will become complete during the fourth day. Anterior and posterior limb buds are now apparent at the levels of somites 17-19 and 26-32 respectively. The tail is curved forward. The fore-gut is still 1.4 mm. in length but has undergone further differentiation, indicated externally by the fact that the first three visceral clefts are open while the fourth is still interrupted by its closing plate. In the branchial arches four aortic arches may be seen. The telencephalon has given rise to the primordia of the cerebral hemispheres, and from the roof of the diencephalon, a small evagination represents the epiphysis or primordium of the pineal gland. The eye and ear, which were formerly in the same transverse section, are now nearly in an antero-posterior relationship. The olfactory pits have made their appearance in the head. The semilunar (fifth cranial nerve), geniculo-acoustic (seventh and eighth), and petrosal (ninth) ganglia may be seen. There are approximately thirty-five somites. The primitive streak has disappeared. The amnion is completed by the fusion of head and tail folds. The allantois, a small sac-like evagination, protrudes ventrally between the posterior limb buds.

Endodermal derivatives. — At the end of the third day the oral aperture has been formed by the rupture of the oral membrane separating the stomodeum and the fore-gut. Immediately anterior to this opening the preoral gut persists. The fore-gut is still the same length as in the chick of forty-eight hours, but is more complex in structure. The thyroid gland, which appeared during the second day, has now become differentiated into the distal dilation which will give rise to the gland proper and the thyroglossal duct. The first three visceral pouches are open to the exterior, but the epithelial buds destined to give rise to the thymus and parathyroids are not yet apparent. The fourth visceral pouch is still separated from the corresponding groove by the closing plate. The laryngeal-tracheal groove has developed in the floor of the pharynx just posterior to the fourth visceral pouches. At its posterior end the dorsal margins of this groove have closed together to form the primordium of the trachea which is thus set free from the esophagus above. The trachea is bifurcated at the posterior end, thus giving rise to the two bronchial buds which are the primordia of the lungs.

The esophagus, which is relatively narrow, is followed by a dilation which is to become the stomach. Posterior to this, the primordium of the liver may be seen as an evagination from the

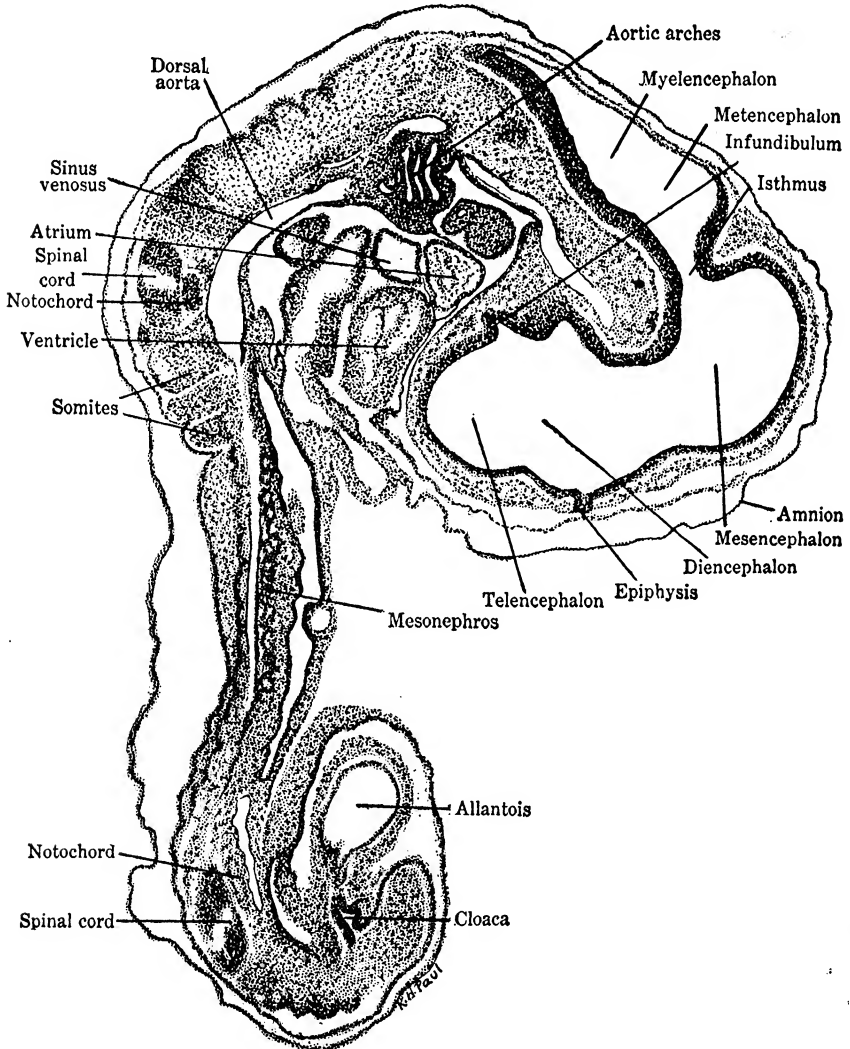


FIG. 219. — 72 hour chick embryo. Sagittal section. $\times 25$.

ventral floor of the duodenal region of the gut. The dorsal pancreas arises from the duodenal region just dorsal to the liver at the end of the third day. The ventral primordia will not appear for another day.

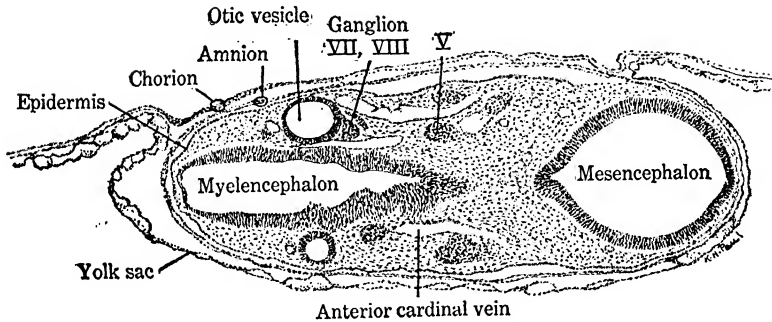


FIG. 220. — 72 hour chick embryo. Transverse section through otic vesicle. $\times 25$.

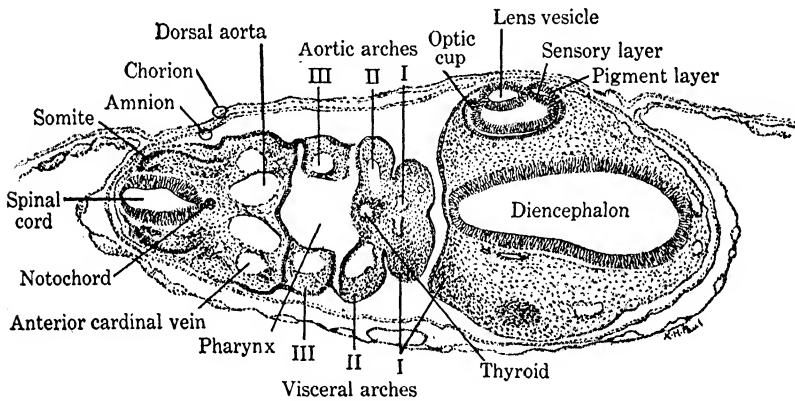


FIG. 221. — 72 hour chick embryo. Transverse section through optic cup. $\times 25$.

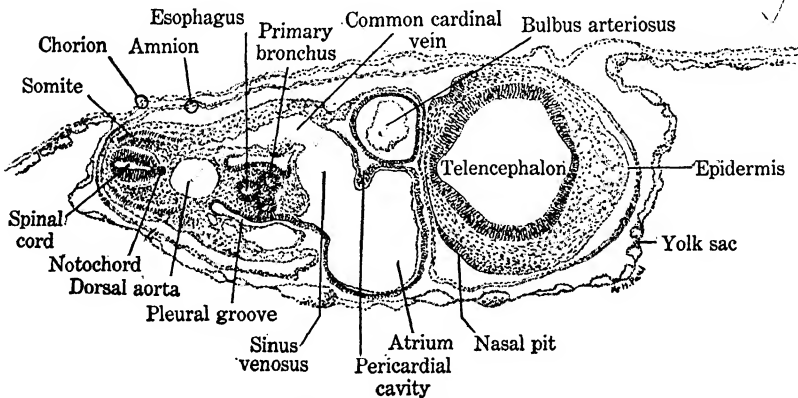


FIG. 222. — 72 hour chick embryo. Transverse section through heart and lung. $\times 25$.

The mid-gut region is gradually lessened by the advancing sulci which are cutting off the body of the embryo from the yolk. This region opens into the yolk stalk which is still quite wide.

The hind-gut contained in the tail fold has not yet acquired its cloacal aperture nor has the proctodeum appeared. The floor of the hind-gut between the tail bud and the posterior intestinal portal evaginates to give rise to the allantoic primordium.

Mesodermal derivatives. — The somites, typically thirty-five in number, still show a varying degree of differentiation which is carried to its furthest point in the more anterior somites. The dermatome is now a thin sheet of cells along the dorso-lateral

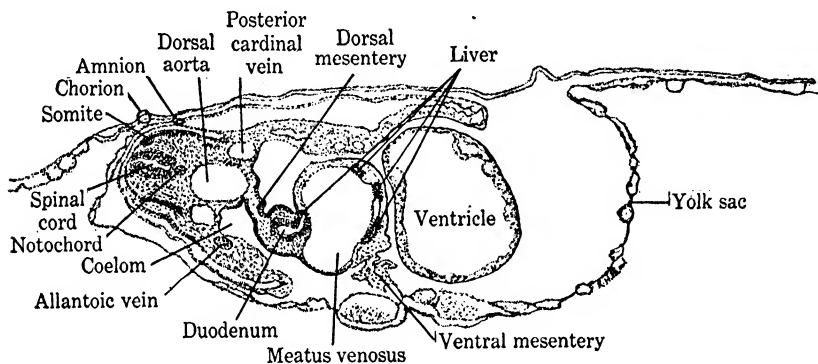


FIG. 223. — 72 hour chick embryo. Transverse section through liver. $\times 25$.

angle of the embryo, with the myotome parallel and internal; the sclerotome in these anterior segments is a large and loose aggregate of cells investing the neural tube, notochord, and aortae.

The pronephric tubules have degenerated to a considerable extent, but the nephrostomes opening into the coelom may persist. The mesonephric tubules are now in process of development, with those in the more anterior segments most highly differentiated. The tubules between the thirteenth and thirtieth somites have progressed from the vesicle stage characteristic of those behind the twentieth somite, and some have acquired a lumen and joined the pronephric duct which henceforward is known as the mesonephric duct. A few of the more anterior tubules develop nephrostomes, but these soon disappear.

Behind the twentieth somite, as far back as the thirtieth, only vesicles are formed. The mesonephric ducts have grown back and united with the cloaca.

The heart now shows a constriction between the atrial and ventricular region. Four aortic arches are developed, of which

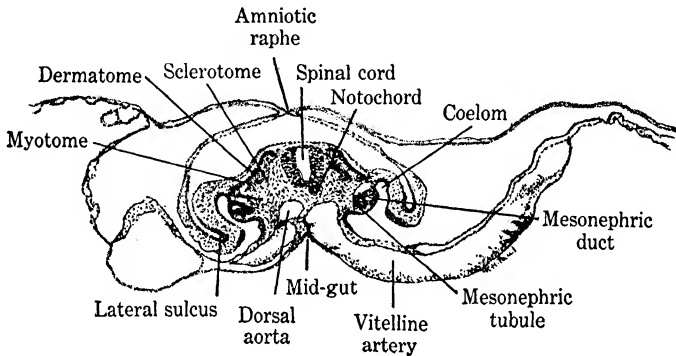


FIG. 224. — 72 hour chick embryo. Transverse section through vitelline arteries leaving body. $\times 25$.

the first is becoming smaller, and sometimes has disappeared at this stage. The internal carotid arteries are now well developed, growing forward into the head from the point of union between the first arches and the dorsal aortae. From the ventral end of the first aortic arch the external carotid takes its origin. The

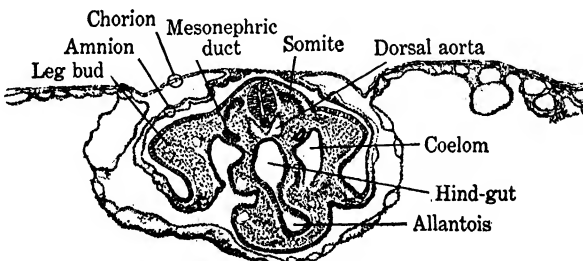


FIG. 225. 72 hour chick embryo. Transverse section through allantois. $\times 25$.

pulmonary is sometimes apparent as a posterior prolongation of the ventral aorta at the point where the fifth arches will appear during the next twenty-four hours. The intersegmental arteries are now apparent as dorsal diverticula from the aorta between each pair of somites. The vitelline veins have fused for a short distance behind the sinus, thus giving rise to the meatus venosus.

The anterior cardinal vein now possesses many branches from the head, among which are three intersegmental veins. The posterior cardinal has continued its backward growth dorsal to the mesonephric duct as far as the thirty-third somite. It receives the intersegmental veins of this region. Where the posterior cardinals unite with the common cardinals, a capillary network indicates the beginnings of the allantoic veins.

Ectodermal derivatives. — The brain at the end of the third day has its five definitive vesicles even more sharply demarcated. From the telencephalon two lateral vesicles have evaginated to form the primordia of the cerebral hemispheres. In the dien-cephalon the epiphysis has appeared as a dorsal evagination. On the floor of this vesicle the infundibulum is almost in contact with the hypophysis. The mesencephalon is separated from the metencephalon by a deep constriction known as the isthmus. Along the sides of the myelencephalon may be distinguished the following cerebral ganglia: the semilunar of the fifth cranial nerve; the acoustico-facialis which will later separate into the geniculate ganglion of the seventh and the acoustic of the eighth; and the petrosal ganglion of the ninth. The eye has increased in size, and the lens is now free from the epidermal ectoderm. The ear, too, is in the vesicle stage and possesses a short endolymphatic duct, which has lost its connection with the epidermis. On the third day the primordium of the nose is represented by two olfactory pits anterior to the mouth.

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CHAPTER XIII

THE ANATOMY OF THE 10 MM. PIG EMBRYO

Pig embryos of 10 to 12 mm. body length are particularly instructive for laboratory work in mammalian embryology as they

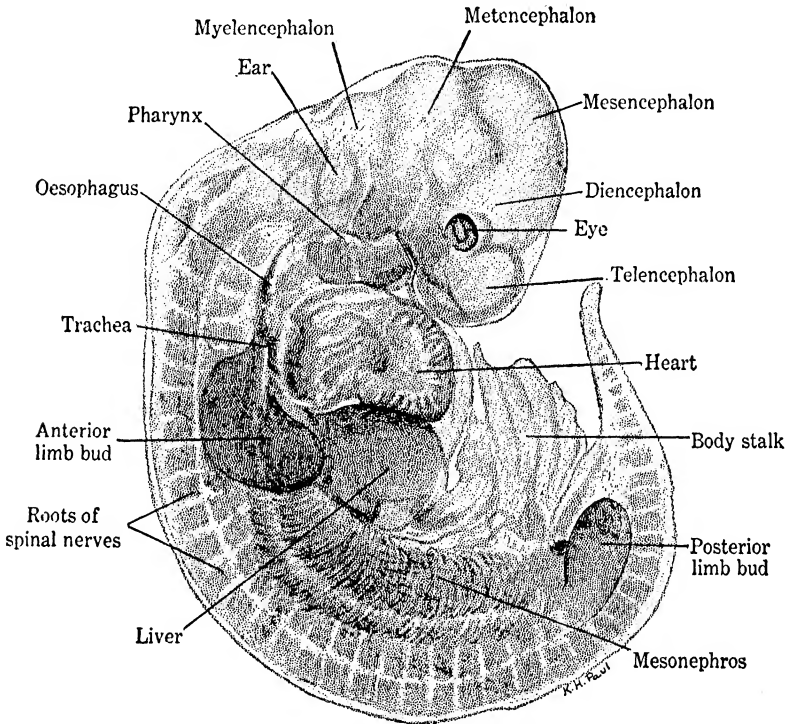


FIG. 226. — 10 mm. pig embryo. Transparent preparation from right side. $\times 11$.

are sufficiently large for the study of external structures and yet small enough to afford serial sections for a detailed study of the anatomy. The primordia of practically all the organ systems, excepting the skeleton and musculature, are present. In comparing the accounts given by different authors of this particular stage, it should be remembered that a large amount of shrinkage takes place during the preparation of fresh sections, so that, as

pointed out by Patten, an embryo of 12 mm. will not measure more than $9\frac{1}{2}$ mm. when prepared for sectioning. The account which follows corresponds in general to the pig (*Sus scrofa*) of 10 mm. described by Keibel, of 12 mm. (Minot), 10 mm. (Prentiss) and 9.4 mm. (Patten), but is not so advanced as the 13.5 mm. pig (Boyden).

External form. — The pig embryo at this stage is relatively more advanced than the chick of seventy-two hours. The body is sharply flexed, owing to the presence of the cranial, cervical, dorsal, and caudal flexures. In the head region the olfactory pits are well developed and are connected by the naso-lachrymal groove to a depression which surrounds the bulging eyeball. The five divisions of the brain are apparent through the relatively thin overlying epidermis. Four visceral grooves can be seen, the first of which, or hyomandibular, is the primordium of the external auditory meatus. The third and fourth grooves are compressed by the cervical flexure into a deeper depression known as the cervical sinus. A frontal view of the head shows the oral cavity bounded above by the frontal process in the middle, the maxillary processes at the side, while the lower jaw is represented by the mandibular arch.

In the trunk region, the buds of the pectoral and pelvic appendages are large but show no further differentiation. The contours of the somites, now forty-four in number, are apparent along the back, and ventral to these can be seen the outlines of the heart, liver, and mesonephros. In some specimens there appears between the limb buds a thickened ridge from which the mammary glands develop and which is therefore known as the milk line. The umbilical cord projects from the ventral side of the embryo. Between this and the base of the slender tail is a small protuberance, the genital tubercle, or primordium of the external genitalia.

Endodermal derivatives. — The preoral gut still persists anterior to the oral aperture. Ventral to this, and seen best in sagittal section, is the long and slender hypophysis, now in contact with the infundibulum of the diencephalon. Both the hypophysis and infundibulum, it should be remembered, are of ectodermal origin. The pharynx is dorso-ventrally compressed, and from its floor the tongue is arising. Four visceral pouches

are present, corresponding to the visceral grooves already noted. These do not unite to become visceral clefts but remain separated by their closing membranes. Between the second and third

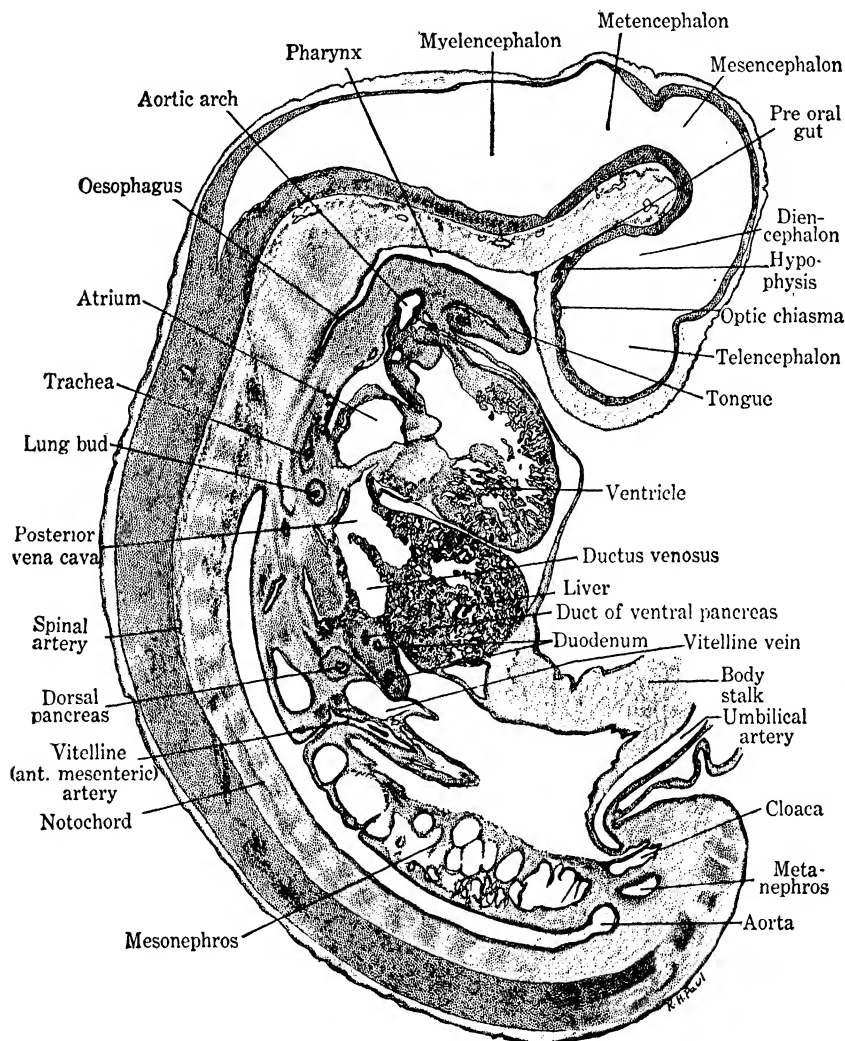


FIG. 227. — 10 mm. pig embryo. Sagittal section. $\times 16\frac{1}{2}$.

pouches the thyroid gland appears. From the level of the fourth pouch a short laryngeal groove is prolonged into the trachea which has given rise to the bronchial buds, three in number. Two of these, the primary bronchi, have arisen by the bifurcation of

the trachea; the third or apical bud, which will give rise to the eparterial bronchus, develops anterior to the right primary bronchus. The esophagus is relatively long and narrow and, just posterior to the level of the lung buds, passes into the stomach which is dilated and shows a slight dorsal curvature. Posterior to the stomach the duodenal glands, liver, and pancreas are well developed. The liver, now a large glandular mass traversed by

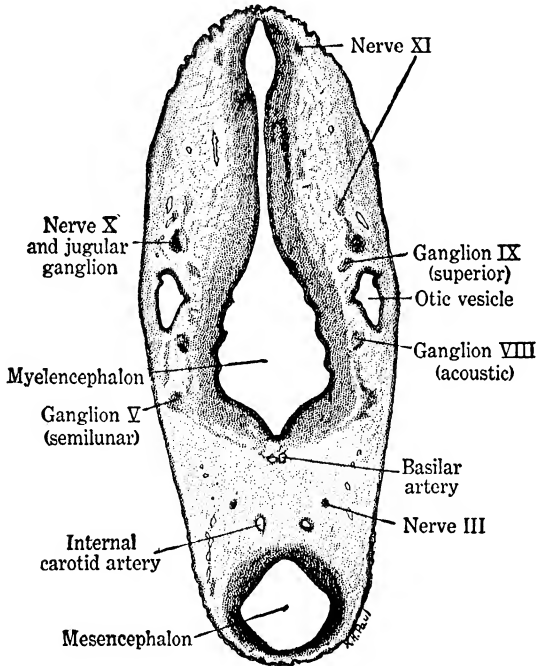


FIG. 228. — 10 mm. pig embryo. Transverse section through otic vesicles. $\times 16\frac{1}{2}$.

the capillaries of the hepato-portal veins, retains its original connection with the duodenum as the common bile duct from the distal end of which the gall bladder is forming. Both dorsal and ventral primordia of the pancreas are present, the duct of the latter arising from the common bile duct. The long and slender intestine extends into the umbilical cord as the intestinal loop, to which the yolk stalk is still attached. Just posterior to this, a slight enlargement may sometimes be observed which indicates the boundary between the large and small intestine. The hind-gut is dividing into a dorsal rectum and ventral urogenital

sinus, prolonged into the allantoic stalk. The sinus and rectum unite in a common cloaca which has not yet established connection with the proctodeum. Immediately posterior to the cloacal plate, a small blind pocket represents the postcloacal gut.

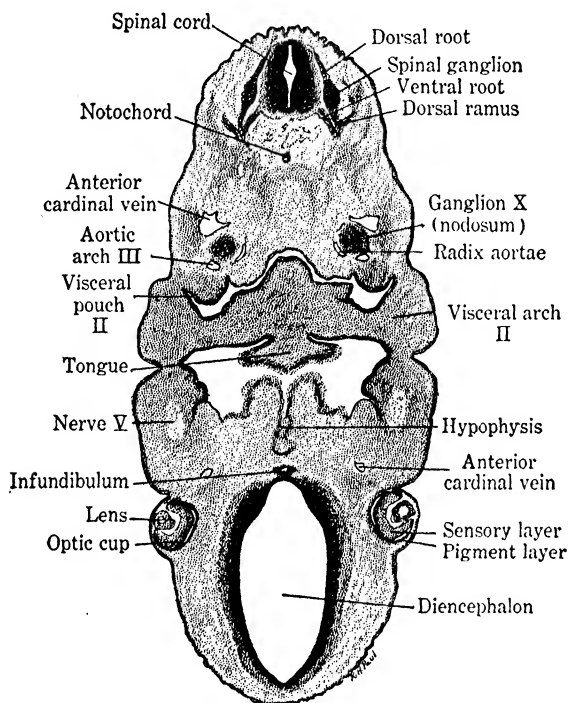


FIG. 229. — 10 mm. pig embryo. Transverse section through optic cup. $\times 16\frac{1}{2}$.

Mesodermal derivatives. — The notochord extends from the vicinity of the floor of the mesencephalon into the tail, following the flexures of the body.

The somites have long since become differentiated into the myotome, dermatome, and sclerotome. In the tail region, the sclerotomes are separated into the cranial and caudal arcualia from which the vertebrae will originate.

In the pig of 10 mm., the pronephric stage has been passed; the mesonephros is at the height of its development, forming a great "Wolffian" body with a complicated network of interwoven tubules; while the mesonephric duct (originally the pronephric duct) may be recognized along the ventral margin. Emerging

from the mesonephros, each duct enters the urogenital sinus at the same level as the allantoic stalk. From each duct a narrow stalk runs dorsally and forward as the metanephric duct, or ureter, which at its distal end is enlarged to form the pelvis of the metanephros. Around the pelvis the posterior portion of the nephrotomal band will produce the secretory tubules of the definitive kidney at a later stage. On the median ventral margin of each

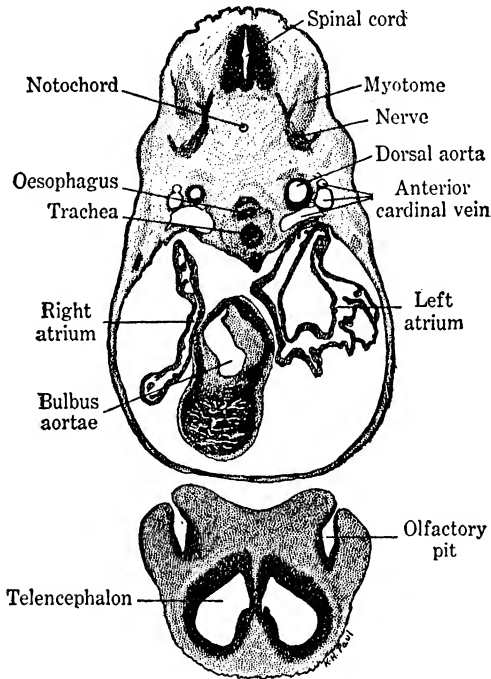


FIG. 230. — 10 mm. pig embryo. Transverse section through nasal (olfactory) pit. $\times 16\frac{1}{2}$.

mesonephros are slight swellings which will later become the genital ridges, primordia of the gonads. The coelom is partially divided into the pericardial and abdominal cavities by the septum transversum. The mesenteries of the principal viscera are in evidence. The liver is still suspended in the ventral mesentery. A dorsal mesocardium is present.

The heart of the 10 mm. pig has the four main chambers established, although not yet completely separated into right and left halves. The sinus venosus now enters the right atrium through

a slit guarded by the valves of the sinus. The right and left atria are partially separated by the interatrial septum in which can be seen an opening, the foramen ovale. The atrio-ventricular canal leading to the ventricle is partially separated into right and left halves by the endocardial cushion. The ventricle is partially divided by the interventricular septum. From the ventral aorta three aortic arches curve around the pharynx to unite with the dorsal aorta. These are the third, fourth, and sixth aortic arches; the first and second have degenerated, while the fifth

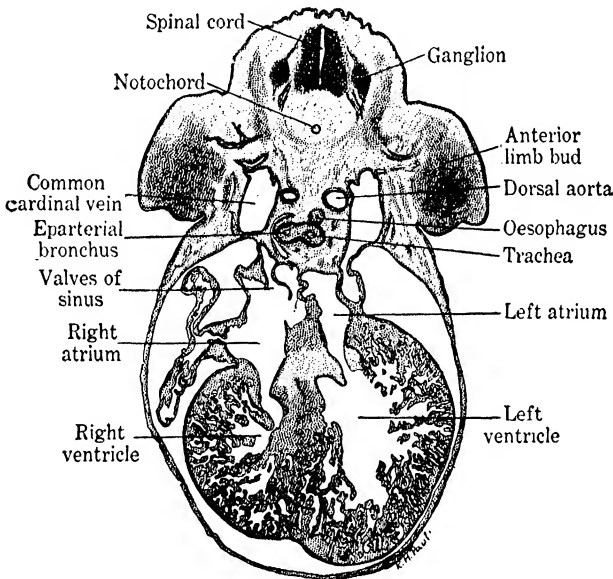


FIG. 231. — 10 mm. pig embryo. Transverse section through sinus venosus. $\times 16\frac{1}{2}$.

seldom appears as a separate structure. The pulmonary arteries are growing back from the sixth aortic arches.

As prolongations of the original paired ventral and dorsal aortae, the external and internal carotid arteries, respectively, run forward into the head. The internal carotid arteries are united at the level of the isthmus between the mesencephalon and the metencephalon with the basilar artery, which serves to unite them with the vertebral arteries, arising from the anastomosis of intersegmental arteries in the cervical region. At the 10 mm. stage the vertebral arteries have lost their intersegmental connections with the aorta except at the posterior end, where the

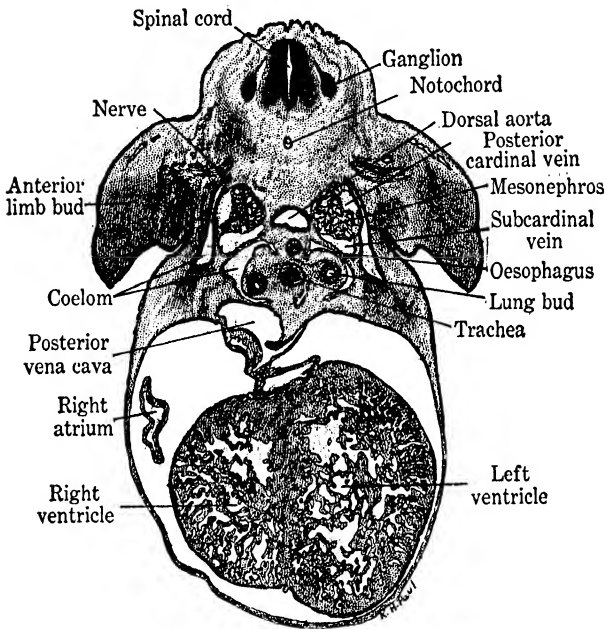


FIG. 232. — 10 mm. pig embryo. Transverse section through lung buds. $\times 16\frac{1}{2}$.

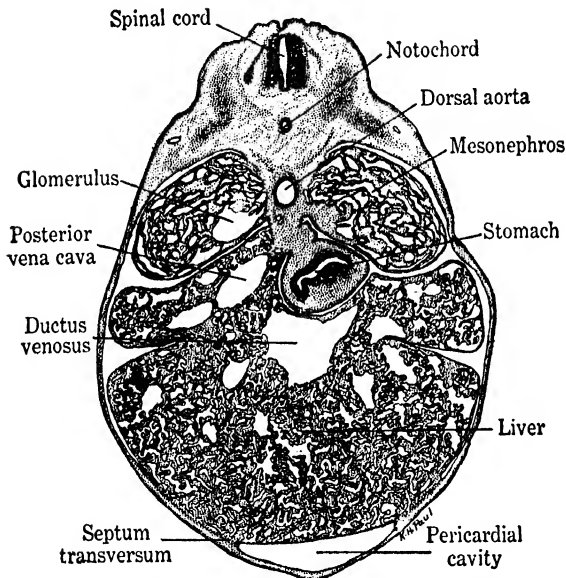


FIG. 233. — 10 mm. pig embryo. Transverse section through stomach. $\times 16\frac{1}{2}$.

seventh cervical intersegmental artery persists and grows out into the pectoral limb bud to form the subclavian artery. Near the point of origin of the subclavian, the dorsal aortae are fused and run back as a single median aorta into the tail. Dorsally, branches are given off from the aorta as intersegmental arteries of the trunk. Laterally, many small branches supply the glomeruli of the mesonephros. Ventrally, the dorsal aorta gives off the coeliac artery and anterior mesenteric arteries to the gut.

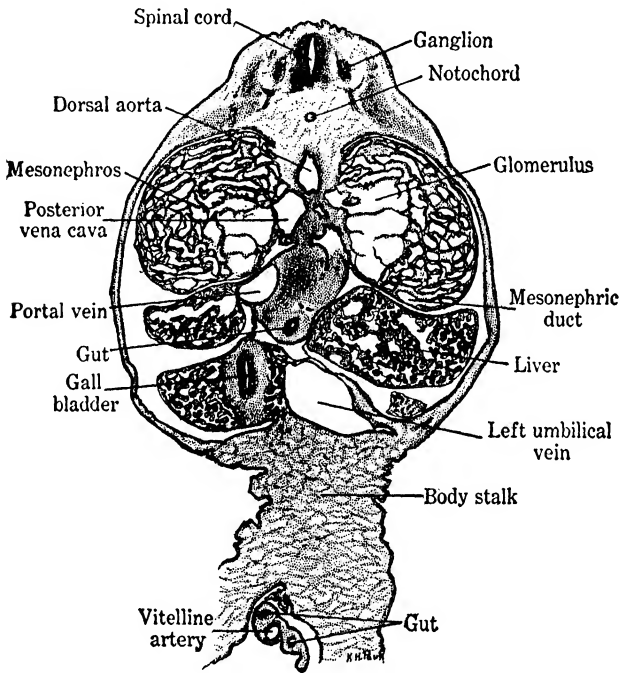


FIG. 234. — 10 mm. pig embryo. Transverse section through gall bladder. $\times 16\frac{1}{2}$.

Two large umbilical (allantoic) arteries run from the dorsal aorta into the umbilical cord. The aorta continues into the tail as a relatively slender vessel, the caudal artery.

The vitelline veins are much smaller than in the chick of seventy-two hours, for the yolk sac from which they drew their blood is nearly degenerated. In the pig at this stage they drain the gut area and cross into the liver where they become the portal vein. Within the liver they are broken up into capillaries which emerge as the hepatic veins to the sinus venosus. Of the somatic

veins, the anterior cardinals are still prominent and are joined by an extensive series of head veins. In the cervical region the anterior cardinals receive the dorsal intersegmental veins as well as the external jugular from the mandible. As the anterior cardinals enter the common cardinal veins, they are joined by the posterior cardinals, which have already lost part of their drainage

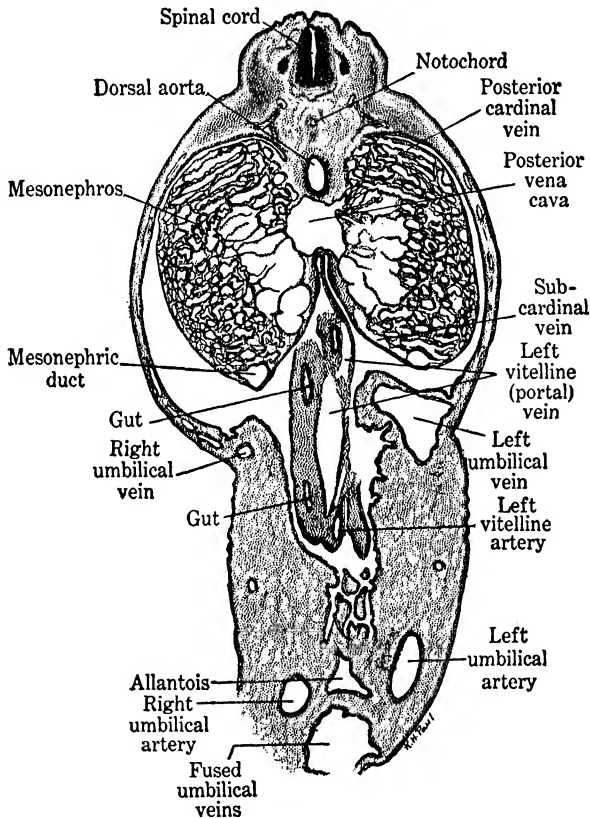


FIG. 235. — 10 mm. pig embryo. Transverse section through umbilical stalk in region of intestinal loop. $\times 16\frac{1}{2}$.

area to the subcardinal veins passing through the ventral portions of the mesonephroi. Numerous small venous channels serve to connect the subcardinals and postcardinals during this period. The posterior caval vein has already made its appearance as a direct connection from the subcardinals to the liver. The umbilical (allantoic) veins proceeding from the allantois toward the heart are fused together in the umbilical cord. In the body they

pass through the liver, within which they are, like the vitelline veins, broken up into capillaries. The left umbilical maintains a broad channel through the liver. This vessel, now known as the ductus venosus, connects the umbilical with the posterior caval vein.

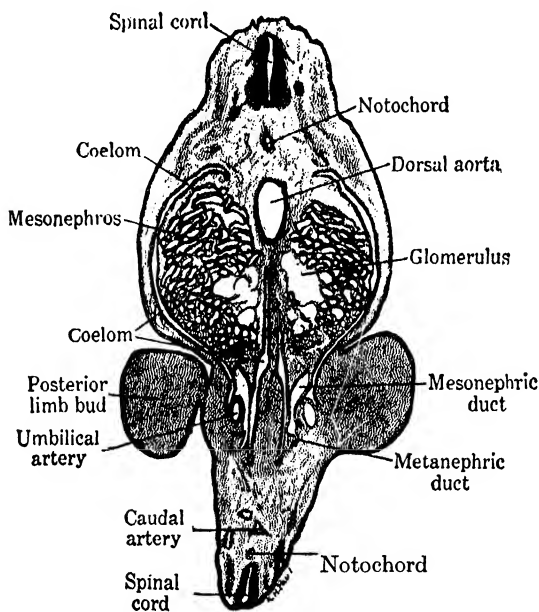


FIG. 236. — 10 mm. pig embryo. Transverse section through metanephric duct and posterior limb buds. $\times 16\frac{1}{2}$.

Ectodermal derivatives. — The epidermal derivatives of the ectoderm have already been enumerated in the description of external form. There remain for consideration the nervous system and sense organs. The five definitive vesicles of the brain are well marked. From the telencephalon arise the two lateral cerebral vesicles. This division of the brain is separated from the diencephalon by two points of reference, the optic recess in the floor, and the velum transversum in the roof. From the diencephalon spring the optic stalks, leading to the optic cups, and the infundibulum, now in contact with the hypophysis as mentioned above. The posterior boundary of the diencephalon is indicated by the tuberculum posterius arising from the brain floor. The epiphysis seldom appears at this stage. The mesencephalon, with the third cranial nerve arising from its floor, is

demarcated at its posterior end by the deep constriction of the isthmus. The metencephalon is distinguished from the myelencephalon by its thicker roof. From the isthmus the fourth cranial nerve runs forward laterally over the sides of the brain to the mass of mesoderm surrounding the eyeball, from which the

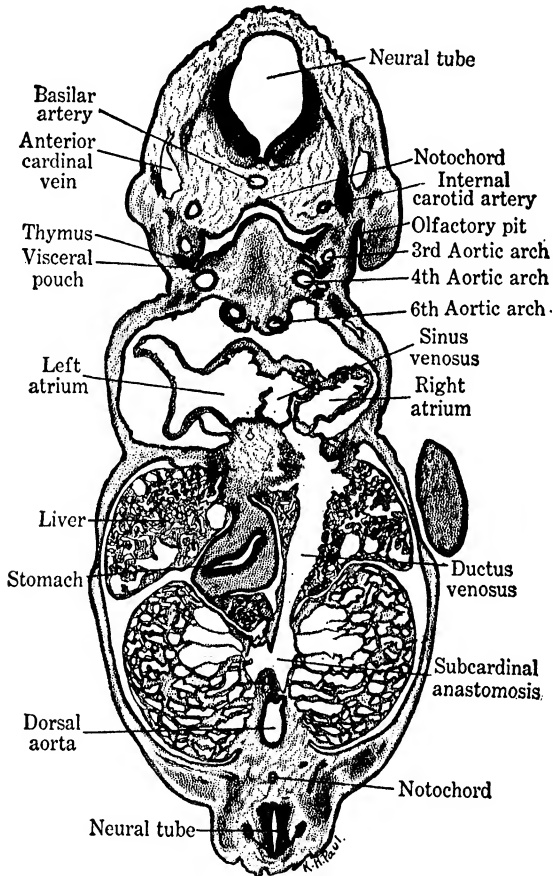


FIG. 237. — 10 mm. pig embryo. Frontal section through aortic arches and ductus venosus. $\times 16\frac{1}{2}$.

eyeball muscles will be formed. Conspicuous at the anterior ventro-lateral margin of the metencephalon is the large semilunar ganglion of the fifth cranial nerve. From the floor of the myelencephalon, the sixth cranial nerve emerges to run forward toward the eye. Immediately following this, the geniculate ganglion of the seventh and the acoustic ganglion of the eighth are in close

connection. The ninth cranial nerve has two ganglia, the dorsal superior ganglion and ventral petrosal, while the tenth similarly possesses a dorsal jugular and ventral nodose ganglion. The eleventh cranial nerve possesses at this stage a small ganglion (of Froriep) which disappears in the adult. The last of the cranial nerves, the twelfth, arises from the floor of the myelencephalon by a number of small roots and without a ganglion. In the region of the spinal cord the segmental nerves arise from the cord by two roots, of which the dorsal is associated with a spinal ganglion. The trunk is very short and soon divides into three main branches. The dorsal and ventral rami run to these respective regions of the body wall, while the third, or communicating ramus, unites the spinal nerve with a ganglion of the sympathetic chain. The sympathetic ganglia may be recognized as small masses of cells dorsal to the aorta.

The nose is represented by the olfactory pits. The eye is in the optic cup stage with a well-marked choroid fissure and groove, while the lens is completely separated from the outer ectoderm and is in the vesicle stage. Of the various regions of the ear, all the primordia are now established. The otic vesicle with its endolymphatic duct, representing the inner ear, is in close juxtaposition to the first visceral pouch (hyomandibular) which will give rise to the auditory tube and chamber of the middle ear; the external auditory meatus, or outer ear, will arise from the first or hyomandibular groove.

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PART V
MICROSCOPICAL TECHNIQUE

CHAPTER XIV

PREPARATION OF EMBRYOLOGICAL MATERIAL

A method much employed in the study of comparative embryology is that of cutting a preserved egg or embryo into a series of extremely thin slices, and arranging these in order upon a glass slide, so that they may be examined under the microscope. The older embryologists, however, were limited to the study of entire embryos and of minute dissections. These methods are still of great value in supplementing the study of serial sections, for it is a difficult mental exercise to translate sections into terms of the whole embryo. The single section, especially, is meaningless except when interpreted as a part of the complete series. It is very helpful, therefore, when facilities permit, for each student to prepare for himself a whole mount and a series of sections through one of the embryos he is to study.

A. COLLECTION AND REARING OF EMBRYOS

Although preserved embryos of the more important laboratory types may be obtained from the biological supply houses, it is often desirable to collect and rear live embryos.

THE FROG. — There are some sixty species of tailless Amphibia within the continental limits of the United States. Although the capture of adults in a pond where eggs are found is strong circumstantial evidence as to the species of the eggs, even this evidence is often lacking, so that the ability to identify the eggs or larvae from their own characteristics is highly desirable. A key to the eggs and larvae of some of the common Eastern frogs and toads is found in Wright's "Life History of the Anura of Ithaca, N. Y." For the Pacific slope fauna, see Storer, "A Synopsis of the Amphibia of California." The eggs of the salamander, *Ambystoma*, are laid at the same time and in the same localities as those of the early frogs, but may be distinguished from them by the greater proportion of jelly to the eggs in the mass of spawn.

Experiments dealing with the effect of pituitary hormones have led to the discovery that one of these hormones will induce ovulation in the female frog, and the drive to amplexus in the male, out of the breeding season. Rugh¹ (1934) has described in detail a technique for inducing ovulation and bringing about artificial fertilization which has been since used in several laboratories, including the author's, with complete success.

The rate of development of the frog's egg depends upon the temperature of the water. In the laboratory, the eggs will hatch in about one week after laying, at the ordinary room temperature. The egg masses should be kept in clean glass containers with at least ten times as much water. The water should not be changed until after hatching, when the larvae should be transferred to fresh water with aquatic plants. After the assumption of the tadpole form, they should be fed small pieces of finely ground meat. Metamorphosis may be hastened by feeding fresh or desiccated thyroid tissue.

Artificial fertilization is the best method of obtaining the earliest stages of development. The testes and vasa deferentia of the male are teased out in a watch glass of water. The eggs from the distal portions of the oviducts are placed in this water for five minutes and then removed to glass containers with not more than four inches of water.

THE CHICK. — In collecting hens' eggs for incubation, it is a truism that they must be fresh and fertile. The best results are obtained from trap-nested eggs in the spring semester. The egg is normally laid in the gastrula stage (Chapter II), but in those cases where the egg does not reach the distal end of the oviduct by 4 P.M., it is retained till the following morning and undergoes further development. After laying, the egg cools and development ceases until incubation is commenced. The fertilized egg is viable for five weeks at a temperature of 8°–10° C. The time of hatching, as in the frog's egg, is dependent upon the temperature. The minimum temperature at which development will take place is about 25° C.; the optimum is 37° C., at which temperature the egg will hatch in twenty-one days; the maximum temperature is about 41° C. In incubating eggs, care must be

¹ R. Rugh. Induced Ovulation and Artificial Fertilization in the Frog, Biol. Bull. 66, 22–29.

taken to keep the air in the incubator moist and to rotate the eggs once a day.

Instructive demonstrations may be made by opening the shell and shell membranes under aseptic conditions and removing a bit of the albumen. A window of celloidin placed over the opening and carefully sealed will permit of observations on the development of the embryo for several days. An alternative method is that of opening the egg and placing the contents in a sterilized small stender dish. A glass ring is placed on the yolk to keep it beneath the surface of the albumen, and the dish is covered and placed in the incubator. If this operation is carried on under aseptic conditions, development will continue for two or three days.

THE PIG. — The early stages of development in any mammal are valuable. The larger embryos are visible as protuberances on the inner side of the uterine tubes. The tube should be slit open and the embryos exposed by cutting open the embryonic membranes which surround them. Smaller stages are obtained by washing out the contents of the tube with normal salt solution or preserving it entire.

Pig embryos may be obtained in quantities from any good-sized packing house. As many as eighteen may be found in a single female, but the average number is eight. The period of gestation in the pig is 121 days. Pig embryos of 10 mm. body length are the most useful in the elementary course. Later stages are of value in the detailed study of organogeny.

B. PRESERVATION OF MATERIAL

The preliminary preparation of material for microscopical work involves three distinct operations: killing, fixing, and preservation. In practice, two or three of these operations are performed by a single reagent known as a "fixing fluid." Such a reagent should kill the embryo so rapidly that it will undergo the minimum of post-mortem changes; it should preserve the structures of the embryo with as life-like an appearance as possible; and it should harden the soft parts so that they may undergo the later processes of technique without loss of form or structure. Some fixing fluids, such as alcohol or formalin, may be used indefinitely as preservatives, but the majority are used for a particular optimum period, and then washed out and replaced by alcohol.

THE FROG. — The frog's egg, *before* hatching, is best fixed by Smith's fluid.

Potassium bichromate.....	0.5 gram
Glacial acetic acid.....	2.5 cc.
Formalin.....	10.0 cc.
Distilled water.....	75.0 cc.

1. Cut the egg masses into small pieces of about twenty-five eggs each, and submerge them in a dish of Smith's fluid for twenty-four hours. A quantity equal to ten times the volume of the eggs should be used.

2. Rinse the eggs in water and wash with a 5 per cent aqueous solution of formalin until no more free color comes out. The eggs may be kept indefinitely in this fluid. If it is desired to remove the egg membranes, proceed as follows:

3. Wash in water for twenty-four hours, changing the water several times.

4. Place the eggs in eau de Javelle, diluted with three times its volume of water, and shake gently from time to time during a period of 15 to 30 minutes until the membranes are almost dissolved and will shake off.

5. Rinse in water and run through 50 per cent and 70 per cent alcohol, an hour to a day each, and preserve in 80 per cent alcohol.

After hatching, larvae are best fixed in Bouin's fluid.

Picric acid, saturated aqueous solution.....	75 cc.
Formalin.....	25 cc.
Glacial acetic acid.....	5 cc.

1. Larvae are left in this fluid from one to eighteen hours, according to size.

2. After rinsing in 50 per cent alcohol, wash in 70 per cent alcohol, to which has been added a few drops of lithium carbonate, saturated aqueous solution, until the yellow color is extracted, and preserve in 80 per cent alcohol.

THE CHICK. — The chick embryo must be removed from the shell, albumen, and yolk before fixation. As the early stages are more difficult to handle, it is advisable to practice this operation on embryos of seventy-two hours' incubation and then work backward toward the stages of the first day.

1. Place the egg in a dish 3 inches high and 6 inches in diameter, two-thirds full of normal saline solution, warmed to 40° C.

Sodium chloride	0.75 gr.
Water	100.00 cc.

2. Crack the shell at the broad end with the flat of the scalpel, and pick away the pieces of shell until an opening slightly larger than a half dollar has been made. Remove the outer and inner shell membranes. Invert egg beneath the surface of the salt solution and allow the contents to flow out. The blastoderm, containing the embryo, will rotate until it is uppermost. With fine-pointed scissors, cut rapidly a circle of blastoderm, about the size of a quarter, with the embryo at the center. With blunted forceps, pull the blastoderm and adherent vitelline membrane away from the yolk and albumen, waving it gently beneath the surface of the salt solution to remove all yolk.

3. Submerge a syracuse watch glass in the salt solution and float the embryo into this. Remove the watch glass carefully from the large dish and examine the embryo with a dissecting lens. If the vitelline membrane has not yet separated from the blastoderm, it should be removed at this time with fine-pointed forceps and needles. Make sure that the embryo lies dorsal side up, as it did when the egg was opened.

4. Slide a cover glass under the embryo, and remove all salt solution with a pipette, taking care that the embryo lies in the center of the cover glass. Lift the cover glass by one corner so that the overhanging edges of the blastoderm fold under, and place it in a dry watch glass on a piece of thin absorbent tissue paper and add fixing fluid at once. While the embryo is becoming attached to the cover glass, remove the yolk, albumen, and pieces of shell from the dish of salt solution to a slop jar, reheat the salt solution to 40° C., and prepare another embryo. Three embryos of each stage are to be prepared.

5. After five minutes, drop the cover glass, embryo side up, into a small stender dish of Bouin's fluid and leave from two to four hours.

6. Rinse in 50 per cent alcohol, wash for two days in 70 per cent alcohol to which lithium carbonate has been added or until the yellow color is extracted from the embryo, and preserve in 80 per cent alcohol.

THE FIG. — Embryos of 6 mm. body length and over are easily located in the uterine wall. Slit open the uterus and remove the embryo with fine-pointed forceps and a horn spoon, taking pains not to rupture the membranes. Place at once in Bouin's fluid. Embryos of 10 mm. body length should be fixed for four hours. Rinsing and preserving are done as for the frog or chick. Larger embryos should have the body cavity slit open to admit the fixing fluid. Fetal pigs of 6 inches or more should be injected through the umbilical artery with formalin (20 per cent aqueous solution). This solution is also injected into the body cavity and cranium, after which the fetus is submerged in the same medium for a week and preserved in 6 per cent formalin.

C. WHOLE MOUNTS

It is very helpful to have some embryos mounted entire for comparison with the serial sections. In making these whole mounts, the embryos are stained, cleared, and mounted, i.e., transferred to a final medium for preservation and examination on the slide beneath a cover glass.

THE FROG. — Frog eggs and embryos may be mounted as opaque objects with the natural pigmentation, or they may be cleared and stained as transparent mounts.

Opaque mounts. —

1. Prepare a saturated aqueous solution of thymol. Filter the solution, and add gelatin until saturated. Remove the supernatant liquid.

2. Liquefy the gelatin by immersing a small quantity, in a test tube, in a dish of hot water. Fill a hollow-ground depression slide with gelatin and allow to cool.

3. With a hot needle, melt a small hole in the gelatin, sufficiently large to hold the embryo. Place the embryo in the desired position and hold it in place until the gelatin has cooled.

4. Add a drop of gelatin just warm enough to be liquid and cover with a cover glass which has been slightly warmed. When the gelatin has cooled, any surplus may be removed from the edges of the cover glass with a toothpick wrapped in moist cotton. In order to prevent the later formation of bubbles, the edges of the cover glass should be painted with gold size or Valspar.

Free-hand sections and dissections are admirably mounted by

this method, but great care must be exercised to prevent the formation of air bubbles through cracks in the gold size.

Transparent stained mounts. —

1. Bleach the embryo, until white, in hydrogen peroxide. About one week is required for this purpose. Embryos that have been preserved in 80 per cent alcohol should first be passed through 70 and 50 per cent alcohol to water, an hour or more in each fluid. Embryos in formalin must be rinsed in water for one hour.

2. Stain in dilute borax carmine four days or more.

Borax, 4 per cent aqueous solution.	100 cc.
Carmine.	1 gr.
Boil until dissolved and add alcohol, 70 per cent.	100 cc.
To dilute, take 5 cc. of the borax carmine and 95 cc. of 35 per cent alcohol and add a crystal of thymol.	

3. If overstained, remove the surplus color with hydrochloric acid (1 per cent solution in 70 per cent alcohol) after passing through water and 50 per cent alcohol, an hour each.

4. Run up through 80, 95, and 100 per cent alcohol, an hour each, and place in xylene (xylol) until transparent.

5. Prepare a mounting diagram by drawing an outline of a slide on a piece of cardboard and in this laying off an outline of the cover glass to be used. Place a clean slide on the diagram, and, just inside the right and left margins of the cover-glass outline, attach a thin strip of celluloid, 15/1000 of an inch in thickness, by means of a drop of acetone. Greater thicknesses may be obtained by attaching other strips as necessary. When these supports are dry, place a few drops of Canada balsam, dissolved in xylene, between the supports, place the embryo in position, and lower a clean cover glass gently. Try to avoid the formation of air bubbles. If these appear later they may be removed by a needle which has been heated or dipped in xylene. A little fresh balsam may be run into the cavity.

THE CHICK. — Total mounts may be stained either with the borax carmine or with Conklin's modification of Delafield's hematoxylin. Delafield's hematoxylin, which gives a blue color to the embryo, is made as follows:

Hematoxylin (16 per cent solution in 100 per cent alcohol).....	25 cc.
Ammonia alum (saturated aqueous solution).....	400 cc.
Hydrogen peroxide, neutralized.....	25 cc.
Glycerin.....	100 cc.
Alcohol methyl.....	100 cc.

Conklin's modification consists of diluting the stain with four times the volume of distilled water and adding to each 100 cc. of the dilute stain 1 cc. of picrosulphuric acid, prepared by adding 2 cc. of sulphuric acid to 98 cc. of picric acid (saturated aqueous solution).

1. Run the embryo from the 80 per cent alcohol down to water through changes of 70 and 50 per cent alcohol, an hour each.

2. Stain in borax carmine, undiluted, over night, or in hematoxylin from one to three hours. Either stain may be diluted still further and the staining period prolonged. In the author's laboratory the schedule demands a four-day staining period and the borax carmine is diluted 5 \times , the hematoxylin 20 \times .

3. Destain, if necessary, in acid alcohol until the desired color is obtained. Embryos stained with hematoxylin will turn red in the acid alcohol, and the blue color must be restored by washing them in running water or, after washing in neutral 70 per cent alcohol, placing them in alkaline alcohol (1 per cent ammonia in 80 per cent alcohol).

4. Run up the alcohols, 80, 95, and 100 per cent, half an hour each. Pour off half the 100 per cent alcohol and add an equal amount of xylene. When the diffusion currents disappear, transfer to pure xylene and leave until the embryo is transparent. In rainy weather, or when 100 per cent alcohol cannot be obtained, phenol-xylene (phenol crystals, 25 gr. and xylene 75 cc.) may be substituted.

5. Remove the embryo from the cover glass (if it has not already detached itself) and trim the surrounding blastoderm to the form of an oblong or circle. Arrange a clean slide on the mounting diagram, as described for the frog, attach celluloid support, and mount the embryo in Canada balsam with the same side uppermost as when the egg was opened. Put the slide away where it may lie flat and free from dust until the balsam has hardened. This will take at least a week, after which the slide

may be cautiously cleaned and studied. The process may be hastened by drying the slide in the paraffin oven.

THE PIG. — Embryos up to 10 mm. body length may be prepared as whole mounts by staining in dilute borax carmine, destaining until only a trace of color persists, and mounting in Canada balsam. The time spent in each alcohol should be at least an hour for the larger embryos.

D. SERIAL SECTIONS

In the preparation of serial sections of an embryo, the fixed material is (1) embedded in a suitable matrix and (2) sliced into extremely thin sections, which are (3) mounted in serial order upon slides. The embryo may be stained before or after sectioning.

Embedding. — There are two principal methods of embedding, in paraffin or in celloidin. For especially delicate objects, the best results are obtained by a combination of these methods, the embryo being first impregnated with celloidin in order to avoid the shrinkage (about 10 per cent) caused by paraffin embedding, and the block of celloidin then immersed in paraffin so that ribbons of serial sections may be cut.

Embedding in paraffin. — In preparing the first few embryos for sectioning, it is advisable to stain, dehydrate, dealcoholize, and clear as if for a total mount. Later, the staining may be omitted until after the sections are affixed to the slide.

1. After clearing in xylene, which should be done in a warm place, for example, the low-temperature oven at about 40° C., pour off half the xylene and add an equal amount of paraffin chips. In the author's laboratory a paraffin of about 55° melting point, obtained by mixing commercial paraffin with parawax, is used. The parawax, unfortunately, varies in melting point, so that the formula is empirical. The embryo may be left in this xylene paraffin for two days.

2. If the mixture has hardened it should again be melted in the low-temperature oven. Fill a clean stender dish with melted paraffin, transfer the embryo to this, and place in the high-temperature oven at about 56° C. (or one degree above the melting point of the paraffin used) for not more than two hours. The xylene paraffin should be thrown in the slop jar. Take care not

to get any xylene in the high-temperature oven or paraffin used for the final embedding.

3. Smear the interior of a small watch glass with a 10 per cent aqueous solution of glycerin (or vaseline), and fill with fresh melted paraffin. Transfer the embryo to this, making any necessary adjustments in position with a heated needle. Place the embryo dorsal side up, and note the position of the head. Cool the surface of the paraffin by blowing on it gently until it is congealed. Then plunge it immediately into a dish of cold water or waste alcohol and leave it there for five minutes. Mark the block for identification. Objects may be left in paraffin indefinitely.

4. On removing the block of paraffin from its container, examine for the following flaws:

a. Air bubbles, if they are not near the embryo, may be removed with a hot needle. Otherwise it is better to trim the block close to the embryo, put it into melted paraffin, and re-embed.

b. Milky streaks are due to the presence of xylene. These will crumble during sectioning, so that it is best to re-embed if they occur near the embryo.

c. If the paraffin has "fallen" in the center, it is because the surface was cooled too long before the block was immersed in the water. If any part of the embryo is exposed, it must be re-embedded.

Sectioning after paraffin embedding. — Before sectioning your first embryo, be sure you understand the mechanism of the microtome (there are many varieties, of which the rotary type is best adapted to beginning students), and have practised the technique on a block of paraffin. There are three standard planes of sectioning corresponding to the axes of the body (Fig. 238). Transverse sections are obtained by cutting the cephalic end of the body first, with the knife entering the left side. Sagittal sections are made by cutting the right side first, with the knife entering the ventral surface. Frontal sections are made by commencing at the ventral surface, the knife entering the left side. It is best to begin with transverse sections.

1. Attach the paraffin block to the object-carrier of the microtome in the proper manner to obtain the type of section desired. This is done by heating the surface of the carrier until it will just

melt paraffin, pressing the block against it in the desired orientation, and lowering into a dish of cold water. A little melted paraffin may be poured around the base of the block and this again cooled to secure additional support.

2. Place the object-carrier in the microtome and, after orienting the block with respect to the knife, trim it so that the end of the block is a perfect rectangle with one of the longer sides parallel to the knife edge. If one of the angles is cut off slightly there will be a series of indentations in the ribbon which will assist in orienting the sections on the slide.

3. If microtome knives are not available, place a new safety-razor blade (Autostrop type) in the holder provided, allowing the

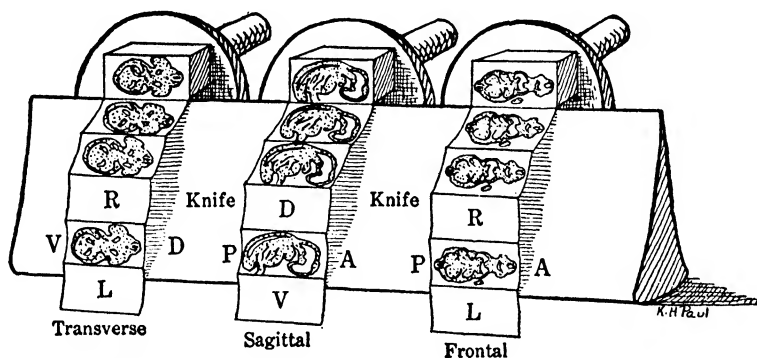


FIG. 238. — Diagram to show method of orienting embryo with reference to microtome knife according to type of section desired.

edge to project between a sixteenth and an eighth of an inch. Screw the holder in the knife-carrier so that the edge of the blade is tilted inward about 10° from the perpendicular.

4. Set the regulator for 20 microns (thousandths of a millimeter).

5. Run the feed screw as far back as it runs freely; do not force it.

6. Advance the knife-carrier until the edge of the blade just clears the block.

7. Release safety catch and turn the wheel steadily until the knife begins to cut the block. Cut slowly, making necessary adjustments to the block and knife until you are cutting a perfectly straight ribbon without wrinkles or splits. The principal causes of trouble and their remedies are as follows:

a. The ribbon curls to right or left. This happens because (1) the block is thicker on the side away from which the ribbon curls, or (2) the knife is duller on the side toward which the ribbon curls. Remedy: (1) trim the sides of the block parallel; (2) shift the knife to one side.

b. The sections curl and the ribbon is not continuous. This is due to (1) too much tilt of the knife, (2) too hard a grade of paraffin, or (3) too cold a room. Remedy: (1) lessen tilt of knife; (2) re-embed in softer paraffin; (3) move microtome to warmer place, light an electric light or micro-bunsen burner near microtome, or cut thinner sections.

c. The ribbon wrinkles badly. This is caused by (1) too little tilt to the knife, (2) too soft a grade of paraffin, (3) too warm a room, or (4) a dull or dirty knife. Remedy: (1) increase the tilt of the knife; (2) re-embed in harder paraffin; (3) move to a cooler room, or cool the knife and block by dropping alcohol on them and blowing vigorously, or cut thicker sections; (4) clean knife edge with cloth moistened in xylene or shift to a new place on the knife.

d. The ribbon splits lengthwise. This is due to (1) a nick in the knife, (2) a bubble in the paraffin, or (3) dirt on the knife edge or side of the block. Remedy: (1) shift to new cutting edge; (2) paint surface with thin celloidin; (3) clean knife edge and block.

e. The sections refuse to ribbon; they fly apart or cling to the knife or the block. This is due to the electrification of the sections caused by unfavorable atmospheric conditions. Many remedies have been suggested; the best is to ground the microtome to a water pipe. Usually it is advisable to wait for more favorable conditions.

8. Remove the ribbon in 6 inch lengths with a camel's hair brush and arrange these in order, shiny side down, in a cardboard box cover. Avoid air currents of all kinds. The ribbons may be put away in a dust-free place if the room is not too warm. It is better to affix them to slides as soon as possible.

Affixing paraffin sections to the slide. — 1. Prepare a mounting diagram by laying off the outline of a slide as before, but enclose in this the outline of a long cover glass (25 by 50 mm. approximately) and leave space for a label on the right-hand side.

2. Clean a slide thoroughly by washing with acid alcohol

followed by distilled water. Place this over the mounting diagram and brush over the surface above the outline of the cover glass with the following dilute solution of egg albumen:

Egg albumen, beaten and skimmed	50 cc.
Glycerin	50 cc.
Filter and add Thymol	a crystal
Dilute 2 drops of this to distilled water	25 cc.

3. Cut the ribbon into lengths about 2 per cent shorter than the length of the cover glass. Using the wet brush from which most of the albumen solution has been squeezed, pick up these lengths and arrange them on the albumenized slide so that the sections will follow each other like the words on a printed page. The shiny side of the ribbon should be next to the slide. Great care should be taken to lower the ribbon slowly so as to prevent the formation of air bubbles beneath it.

4. Carefully warm the slides on a warming plate or a piece of plate glass, previously heated in the paraffin oven, until the sections are expanded and perfectly smooth. If bubbles appear beneath the ribbon, prick them with a hot needle while the ribbon is still soft and hot. Drain off the surplus water, carefully realign the sections, mark the slides with a glass-marking crayon, and set them away in the low-temperature oven to dry, at least two days. They may be kept indefinitely in this condition if not exposed to dust.

Embedding in celloidin. — This method is preferred by some technicians as no heat is used in the process and the shrinkage is less than that resulting from the paraffin method. However, thin sections are not so easy to obtain and the sections must be handled individually.

1. Embryos are dehydrated as for the paraffin method. Leave in absolute alcohol one day.

2. Absolute alcohol and ether, equal parts, one day.

3. Thin celloidin, three days to one week.

Alcohol, 100 per cent	100 cc.
Ether	100 cc.
Celloidin	5 gr.

4. Thick celloidin, two days to two weeks.

Alcohol, 100 per cent	100 cc.
Ether	100 cc.
Celloidin	15 gr.

5. Remove the embryo to a small watch glass and pour thick celloidin over it. Cover lightly, or place under a bell jar until the celloidin is hard enough to cut with a scalpel.

6. Dip a block of vulcanized fiber in thick celloidin. Cut out a block of celloidin containing the embryo from the watch glass and, after moistening the end by which it is to be attached in ether alcohol, press it firmly against the prepared fiber block.

7. Pour a little chloroform into a stender dish, add the block and embryo, cover tightly, and allow the celloidin to harden in the fumes for thirty minutes.

8. Fill the stender dish with chloroform and cover. Leave for thirty minutes.

9. Pour off half the chloroform and add an equal amount of cedar oil. Leave for one hour.

10. Transfer to pure cedar oil where it may remain indefinitely.

Sectioning after celloidin embedding. — Celloidin sections are usually cut with some form of sliding microtome. Be sure to study the mechanism and cut a piece of hardened celloidin before proceeding further.

1. Set the knife with a little more tilt than would be used for paraffin, and obliquely to the object so that at least half the cutting edge will be drawn through the block.

2. Orient the block upon the object-holder so that the desired type of sections may be obtained. The long side of the block should be parallel to the edge of the knife.

3. Cut sections 20 μ or more in thickness, using a steady drawing cut. Mount sections as they are cut.

Affixing celloidin sections to the slide. — This is best done as the sections are cut.

1. Using the mounting diagram as before, rub on a thin film of undiluted albumen solution to cover the areas of the cover glass. Rub in well with the ball of the finger.

2. Arrange the sections in order on this area. When this is filled, lay a cigarette paper over the sections and press gently with another slide. The slides may be kept in a dust-free container.

Double embedding in celloidin and paraffin. — This process, although tedious, combines the best points of the two methods already given.

1. Embed in celloidin according to the method above, omitting step 6.

2. Trim the celloidin block close to the embryo and wash out the cedar oil with xylene, three changes in two hours.

3. Embed in paraffin as described above, commencing at step 2.

4. Section according to the method given for paraffin.

5. Affix to the slide according to the method given for paraffin sections.

Staining serial sections. — When the embryo has been stained before sectioning, it is only necessary to remove the paraffin (or celloidin), replace with Canada balsam, and cover, if the stain proves to be satisfactory. Sometimes, however, it is advisable to strengthen or weaken the stain or to add a contrasting dye.

After staining in bulk. —

1. **Paraffin sections** on the slide should be put in a Coplin staining jar of xylene and left until the paraffin is dissolved, up to fifteen minutes.

2. Transfer to a mixture of xylene and 100 per cent alcohol, equal parts, five minutes.

3. Transfer to 100 per cent alcohol, five minutes.

4. Examine slide rapidly under microscope after wiping the back of the slide.

a. If the stain is satisfactory:

5a. Absolute alcohol and xylene, five minutes.

6a. Xylene, ten minutes.

7a. Mount in balsam under cover glass.

b. If the stain is too intense:

5b. Ninety-five and 85 per cent alcohol, one minute each.

6b. Acid 70 per cent alcohol, until stain is correct.

7b. Sections stained in hematoxylin should have the blue color restored in alkaline 85 per cent alcohol.

8b. Eighty-five, 95, and 100 per cent, one minute each.

9b. Absolute alcohol and xylene, five minutes.

10b. Xylene, ten minutes.

11b. Mount in balsam.

c. If the stain is too light:

5c. Ninety-five, 85, 70, and 50 per cent alcohol, one minute each.

- 6c. Stain until desired effect is secured.
- 7c. Distilled water, five minutes.
- 8c. Fifty, 70, 85, 95, 100 per cent alcohol, one minute each.
- 9c. Absolute alcohol and xylene, five minutes.
- 10c. Xylene, ten minutes.
- 11c. Mount in balsam.

Celloidin sections on the slide should be exposed to the fumes of the alcohol-ether mixture for half a minute, dried for one minute, and placed in a staining jar of 95 per cent alcohol. All other operations may be carried on as above except that phenol-xylene should be substituted for 100 per cent alcohol.

Counterstaining after staining in bulk. — In order to differentiate the parts of the embryo more sharply, it is often desirable to add a second stain contrasting with the first. The stains that have been employed in the previous exercises are nuclear dyes; that is, when extracting by acid alcohol, the color will persist in the nucleus after it has been washed out of the cytoplasm. The second stains affect the cytoplasm and should contrast in color with the nuclear stain employed. After borax carmine, a 0.5 per cent solution of anilin (Lyons) blue in 95 per cent alcohol is employed; after hematoxylin, a similar solution of eosin should be used.

1. Proceed as in the preceding section as far as 6b.
2. Destain in acid alcohol until the color persists only in the nuclei.
3. Restore the blue color to hematoxylin-stained sections in alkaline 80 per cent alcohol.
4. Eighty and 95 per cent alcohol, one minute each.
5. Counterstain lightly, dipping the slide into the solution repeatedly until a light color persists in the sections, one-half to one minute.
6. Rinse in 95 per cent alcohol, dehydrate with 100 per cent alcohol, followed by xylene-absolute, clear in xylene, and mount.

Staining with Delafield and eosin on the slide. — Follow directions given for sections stained in bulk (where stain is too light), as far as step 6c, and follow with directions for counterstaining as given above.

Staining with Heidenhain's hematoxylin. — This is one of the most important embryological stains.

1. Remove the paraffin from the sections and run down the alcohols to distilled water.

2. Four per cent aqueous solution of iron alum, one hour to over night.

3. Rinse in distilled water and place in 0.5 per cent aqueous solution of hematoxylin, same time as in the iron alum.

4. Rinse in distilled water and return to the iron alum until sections are a pale gray. Check from time to time by rinsing in distilled water and examining under microscope to see that the desired structures are still visible.

5. When sufficiently destained, wash in running water for twenty minutes, or in distilled water, with frequent changes, for two hours.

6. Run up the alcohols, clear, and mount.

Fuchsin and picro-indigo-carmin. — This polychromatic stain is especially fine for organogeny.

1. Remove the paraffin and run down the alcohols to distilled water.

2. Stain in basic fuchsin, saturated aqueous solution, twenty minutes.

3. Rinse in distilled water and place in picro-indigo-carmin for five minutes.

Picric acid, saturated aqueous solution 50 cc.

Indigo-carmin, saturated aqueous solution 50 cc.

4. Pass rapidly through 70, 95, and absolute alcohol into xylene-alcohol. The green dye is extracted most rapidly by the 70 per cent alcohol, the red by the absolute. Only experience will teach the right time allowance for each alcohol.

5. Clear in xylene and mount.

Oppel's polychromatic stain. — This gives beautiful effects with older embryos and larvae.

1. Fix in Bouin.

2. Stain in bulk with undiluted borax-carmin, one to two days. Destain for the same period.

3. Embed, preferably by the double method.

4. Cut sections, 15–20 μ .

5. Run down the alcohols to water.

6. Stain in picro-indigo-carmin, 1½ minutes.

7. Stain in picro-fuchsin, one minute.

Picric acid, saturated aqueous solution.....	50 cc.
Acid fuchsin, saturated aqueous solution.....	50 cc.

8. Wash in distilled water, changed repeatedly, five minutes.
9. Ninety-five per cent alcohol, two minutes.
10. Phenol-xylene, xylene, and mount.

E. TECHNICAL RECORDS

Not the least important part of technique is the keeping of exact records covering every technical operation. For each embryo there should be a card, giving the following data:

1. Kind of embryo and stage of development.
2. Method of fixation, time and date.
3. Bulk staining, time and date.
4. Method of embedding, time and date.
5. Plane and thickness of sections, and date.
6. Slide staining, time and date.
7. Method of mounting, and date.
8. Name of preparator.

F. OUTLINE OF TECHNICAL PROCEDURE FOR CHICK EMBRYOS

1. Remove embryo from egg in warm normal salt solution.
2. Fix for two hours in Bouin's fluid.
3. Wash in 70 per cent alcohol (plus lithium carbonate), at least one change, for two days.
4. Pass through 50 per cent alcohol and water, one hour each.
5. Stain in dilute borax-carminc or Delafield's alum-hematoxylin, four days.
6. Destain in acid 70 per cent alcohol until desired effect is obtained.
7. Wash in neutral 85 per cent alcohol. (The hematoxylin-stained specimen is transferred to alkaline 85 per cent alcohol until blue color is restored.) Two days.
8. Dehydrate and clear: 95 per cent, 100 per cent alcohol, absolute alcohol-xylene, xylene, twenty minutes each.

Mount in Canada balsam

OR

9. Prepare for embedding by pouring off half the xylene and adding an equal amount of paraffin chips. Keep in warm place up to four days.

10. Continue by transferring embryo to melted paraffin and place in paraffin oven for an hour and a half.
11. Embed in fresh paraffin and cool in water. Make blocks.
12. Cut transverse sections 20 μ in thickness on microtome.
13. Prepare clean albumenized slide, float sections on this in order, warm until sections are expanded, remove surplus water. Dry for at least two days.
14. Remove paraffin with xylene, and
 - A. Mount in balsam, or
 - B. Run down alcohols to 70 per cent and destain. Run up the alcohols, through absolute alcohol and xylene and xylene, mount in balsam, or
 - C. Run down alcohols to water and restain, dehydrate, clear and mount, or
 - D. To 95 per cent and counterstain for one minute. Dehydrate, clear, and mount.

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CHAPTER XV

STUDY OF EMBRYOLOGICAL PREPARATIONS

During the early stages of development, embryos are too small to be studied with the unaided eye. Some observations, to be sure, may be made with the dissecting lens, but most embryological work requires the use of the compound microscope. Although the student may be familiar with the use of the microscope from the elementary course in biology, he should nevertheless review this subject before proceeding further. In addition, he should at this time familiarize himself with the simpler methods of measuring objects with the aid of the microscope, as embryological drawings require a strict accuracy as to proportions. A great convenience in embryological work is the camera lucida or some other device by means of which accurate outlines may be traced. Finally, we must consider the methods by which the embryo may be reconstructed in magnified form from serial sections, thus returning, in a sense, to the point where the study of embryological technique was begun.

A. THE USE OF THE MICROSCOPE

Nomenclature of the microscope. — The separate parts of the microscope (Fig. 239) may be grouped into two systems, the mechanical parts, and the optical parts. The principal mechanical parts are the base, from which arises the pillar, attached to which is the arm, which may be inclined at the joint. Attached to the arm, just above the joint, is the stage, upon which the slide is placed for examination, and beneath this, the movable sub-stage equipment, consisting of a condenser-sleeve, and one or two iris-diaphragms, by means of which the amount of light to be used is regulated. At the base of the arm is the mirror, a silvered double mirror, with a plane surface on one side and a concave surface on the other. At the upper end of the arm are two screws, the coarse and fine adjustments, by means of which the barrel of the microscope may be raised or lowered either

rapidly or very slowly. The barrel is composed of the body-tube, connected to the arm by a rack and pinion, in the upper end of which is enclosed an inner tube, the draw-tube, on which is a graduated scale of millimeters representing the tube length exclusive of the revolving nose-piece at the lower end. The optical parts of the microscope are systems of lenses, the condenser, placed in the condenser-sleeve, the objectives, attached to the revolving nose-piece, and the oculars, one of which is placed at the upper end of the draw-tube.

The condenser. — This is a system of lenses which increases the amount of illumination thrown upon the object, and is required only with the higher-power objectives.

The objectives. — These are systems of lenses which produce an enlarged and inverted image of the object under proper conditions. Objectives were formerly marked by arbitrary letters or numbers, with the lowest-power objectives beginning the series.

To-day they are usually indicated by the equivalent focal length (E. F.), that is, the focal length of a simple lens at 250 mm. or 10 inches, or else by the actual magnification (\times) at 160 mm. (Leitz microscopes, 170 mm.). In some of the older microscopes the tube lengths indicated on the draw-tube were calibrated without including the length of the revolving nose-piece, then an accessory part. When setting up these instruments the length of the nose-piece (Leitz, 18 mm.) must be deducted and the draw-tube set at the reduced length (Leitz, 152 mm.). The most useful objectives for general embryological purposes are the 25-mm. or 6 \times , which will hereafter be spoken of as the lower-power objective; the 16-mm. or 10 \times , which will be called the

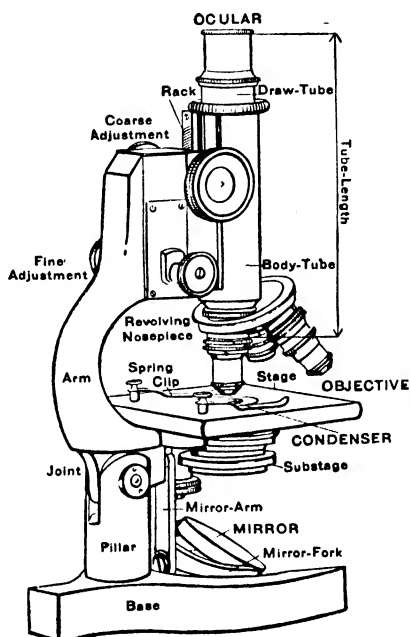


FIG. 239. — Diagram showing parts of the compound microscope. (From Gage.)

medium-power objective; and the 4-mm. or $40\times$, known as the high-power objective. For the study of the germ cells, an oil-immersion objective, of which the front lens must be in contact with the cover glass by means of a drop of cedar oil, is necessary. The most generally used immersion objective is that of 1.9 mm. E. F. or approximately $95\times$.

Oculars. — These are systems of lenses which magnify the real image formed by the objective. Like objectives, these were, in the past, usually numbered or lettered, beginning with that of the lowest power, but now are marked with the E. F. at 250 mm. or the actual magnification at 160 mm. (Leitz oculars, 170 mm.). The most useful oculars are the 50-mm. ($5\times$) or low-power ocular, and the 25-mm. ($10\times$) or high-power ocular. When used with the objectives given above, a range of magnification from $30\times$ to $450\times$ may be obtained. A method of obtaining the exact magnification will be described in connection with the directions for reconstruction given below.

The use of the microscope. —

1. Place the microscope squarely in front of you with the pillar toward you and the stage horizontal.

2. Place the low-power ocular in the draw-tube, and adjust this to a length of 160 mm. (170 mm. for Leitz instruments) as indicated on the millimeter scale. Swing the low-power objective into position. Place the mirror bar in the median line and adjust the mirror to secure an even illumination. Use the plane side of the mirror. The concave side is employed only when the condenser is not in use.

3. Place the slide on the stage so that the object to be examined is in the center of the stage aperture, and fasten it down with the spring clips provided. With the coarse adjustment, lower the body-tube until the objective nearly touches the cover glass. Then, with the eye at the ocular, slowly raise the body-tube until the object comes into plain view. With the fine adjustment, raise and lower the body-tube a little at a time until the point at which the smallest details show clearly is discovered. This is the focal point.

4. When using the low-power and medium-power objectives, the condenser should be lowered until the illumination is evenly distributed. With the high-power objective, the condenser should

be raised almost to the level of the stage. The iris diaphragm should be open sufficiently to illuminate about three-quarters of the aperture of the objective. In other words, it is more widely open for the low-power objective than for the high-power objective.

5. If a greater magnification is desired, change to the high-power ocular, which will double the magnification. If this is not sufficient, return to the low-power ocular and swing the medium-power objective into position, and so on. On most modern instruments, the objectives are par-focal; that is to say, the lengths of the objectives are such that when another objective is swung into place the object will still be visible. If, however, the object is not in focus, it is best to lower the body-tube until the new objective almost touches the cover glass, and focus up until the object comes into view. If the oil-immersion objective is to be used, lower the condenser and place a drop of oil on its upper surface; then raise it until it touches the bottom of the slide. Place another drop immediately over the object on the cover glass and lower the body-tube with great care until the front lens of the objective touches the oil. Focus by means of the fine adjustment only.

6. All optical parts of the microscope must be cleaned with lens-paper. After the oil-immersion objective has been used, the front lens, condenser, and slide should be wiped with a bit of lens-paper dipped in xylene and then dried with a fresh piece. Never separate any of the optical parts. The microscope should be lifted by the pillar unless a special grip is provided to the arm. The microscope should be kept in the case when not in use. One of the oculars should be left in the draw-tube at all times to prevent dust getting on the upper lenses of the objectives. Beginners should try to avoid the error of closing the eye that is not in use. Practice will enable the microscopist to work with both eyes open and even to alternate the right and left eye at the ocular.

Micrometry. — The unit of measurement in microscopy is the micron (μ). It is the one-thousandth part of a millimeter. Measurement of microscopic objects is performed with the aid of micrometers, of which there are two types, the stage micrometer and the ocular micrometer. The former is a glass slide, in the center of which, under a cover glass, is a line, usually 2 mm. long,

divided into 200 equal parts, each of which, therefore, is equivalent to 10 μ . The ocular micrometer is a glass disc, placed in an ocular at the level of the ocular diaphragm, on which is engraved a scale, with arbitrary subdivisions. Some oculars are furnished with a draw-tube so that the upper lens of the system may be focused more sharply upon the scale. The value of the divisions indicated on the scale varies according to the amount of magnification of the real image, and so must be obtained for each objective independently, according to the following method:

1. Arrange the microscope as before, taking particular care to secure the proper tube-length.

2. Focus the eye-lens on the ocular micrometer scale by means of the ocular draw-tube. Focus the objective on the stage micrometer.

3. Make the lines of the stage micrometer parallel with those of the ocular micrometer, and determine the value of the divisions of the ocular micrometer in terms of those of the stage micrometer. Thus, if it requires 10 spaces of the ocular micrometer, and the latter is equal to 0.1 mm., then the value of a single space of the ocular micrometer for that particular objective and at that particular tube-length is 0.01 mm. or 10 μ . Determine the value of the ocular micrometer for each objective in the same way.

B. EMBRYOLOGICAL DRAWINGS

Free-hand drawings of microscopic objects can only approximate an accurate representation. However, great pains should be taken to secure at least accurate proportions, neat and clean-cut lines, and complete labels. Accurate outlines can be secured by the aid of the camera lucida, various types of projection apparatus, or microphotography.

Equipment. — The student will need a hard lead pencil (4H), a medium pencil (HB), and blue, red, and yellow colored pencils, an eraser, and bond paper to fit the note-book cover used in earlier courses.

Free-hand drawing. —

1. Lay off the space to be occupied by the drawing, by placing four dots at the corners. Rule in two lines, intersecting at the center of this space. These will represent the dorso-ventral

and the dextro-sinistral axes, if the drawing is to be of a transverse section.

2. Measure the corresponding axes of the sections by means of the ocular micrometer, multiply by the desired magnification of the drawing, and lay off these magnified measurements on the cross lines already drawn. The following magnifications are recommended: for the twenty-four hour chick, $100\times$; for the thirty-three hour chick, $75\times$; for the forty-eight hour chick, $50\times$; for the seventy-two hour chick, $30\times$; for the 10 mm. pig, $20\times$.

3. Draw in a careful outline of the section and of the internal structures, paying particular attention to the proportions, which should be measured with the ocular micrometer and laid off on the axes at the proper magnification.

4. On one side of the dorso-ventral axis, all structures should be colored with the crayons in accordance with the following scheme: ectoderm, blue; mesoderm, red; and endoderm, yellow.

5. Label all structures represented in the section, using broken lines at right angles to the long axis of the paper to connect the label with the structure indicated.

6. Identify the drawing fully, by means of a serial number, the species, and stage of development, the number given to the series, slide, and section, the type of sections, and the amount of magnification. Example: No. 23, Chick, 48 hours, Series 1102, Slide 2, section 28, transverse section $50\times$. If a drawing has already been made of the total embryo or a total mount, indicate on this, by means of a heavy ruled line, the position of the section just drawn, and number this line with the serial number of the section.

Abbé camera lucida. — This is an attachment which reflects the light from the drawing board, by means of a mirror, to a silvered prism, whence the light is reflected to the eye, superimposed on the image of the object which is transmitted through a small hole in the silvered surface of the prism directly above the ocular of the microscope (Fig. 240).

1. Attach the camera to the draw-tube of the microscope in such a way that the mirror projects to the right, and the opening in the prism lies above the center of the ocular.

2. Extend the mirror arm to its greatest length and set the

mirror at an angle of 45° . The mirror arm must be parallel to the drawing board.

3. Try various combinations of objectives and oculars until an image of the desired magnification appears on the paper. Magnifications intermediate to those ob-

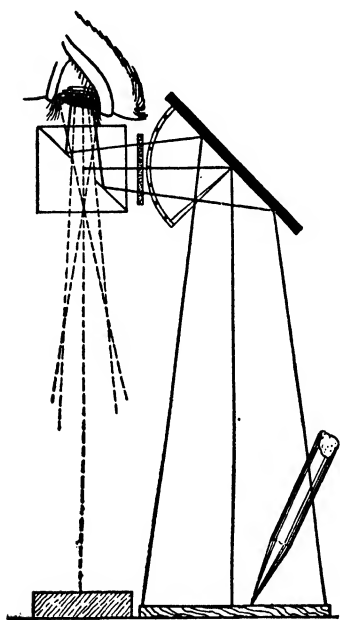


FIG. 240. — Diagram showing principle of the Abbé camera lucida. Path of image seen in microscope shown in broken lines, that on drawing paper shown in unbroken lines. (From Gage.)

tainable in this way may be secured by varying the tube-length or by raising or lowering the drawing board. If the stage of the microscope interferes with the drawing, the mirror should be set at an angle of 40° or 35° and the drawing board tilted toward the microscope at an angle of 10° or 20° , respectively, by means of wooden images. If the image is stronger than the reflection of the pencil point, a smoked glass may be placed beneath the prism, or the aperture of the iris diaphragm may be reduced. If the reflection of the pencil is stronger than the image, smoked glass may be placed at the side of the prism or the amount of light falling on the paper reduced by means of a screen.

4. Draw in the outlines of the sections and the larger internal structures. The details may be added free-hand.

5. Remove the slide and substitute a stage micrometer. Trace in part of the scale by means of which both the magnification of the drawing and the absolute size of the object may be computed readily.

Projection apparatus. — Where many drawings are to be made, as in the case of reconstructions, some form of apparatus by means of which the image of the section may be projected directly upon the paper is very helpful. There are many types of projection apparatus, directions for the use of which may be obtained with the instruments.

Microphotography. — The photography of minute objects with the aid of the microscope is of great assistance in embryology. However, the methods are so difficult, the apparatus so complex, expensive, and delicate, and the process requires so much technical knowledge and skill, that microphotography has been considered a field too advanced for the beginning student, although a method described by Headland seems to overcome these difficulties to a large extent. In recent years the motion-picture camera has been adapted for use with the microscope, and excellent results have already been obtained.

C. RECONSTRUCTION

After an embryo has been sectioned, it is sometimes necessary to reconstruct some part of it from the sections. There are two important methods: graphic reconstruction, in which a geometric projection of a sagittal section, for example, might be made from transverse sections; and plastic reconstruction, in which magnified replicas of each section are made of wax and piled together so as to make an enlarged model of the object to be studied. A complete series of sections of uniform thickness and accurate orientation is required for either type of reconstruction, and an outline drawing of the embryo before sectioning is of great assistance.

The graphic method (of His). — This method can best be described by giving practical directions for a particular problem, e.g., to prepare a geometrical sagittal projection $20\times$ of the neural tube of a 10 mm. pig embryo from a series of transverse sections $20\ \mu$ in thickness.

1. From the lateral view of the embryo drawn before sectioning, make an outline drawing $20\times$.

2. Draw a median line corresponding to the cephalo-caudal axis, the length of which, in this case, should be 200 mm.

3. Count the number of sections in the series, in this case, 500.

4. Locate the position of each transverse section which you have drawn on the median line of the outline. Thus if the most anterior section drawn was the fiftieth of the series of 500 sections, it would be located at a point $1/10$ of the total length of the axis ($200\ \mu$), or 20 mm. from the cephalic end.

5. Theoretically, each section is at right angles to the median

line, but this angle may be greater or less as a result of variations in technique. Study each drawing of a cross section in connection with the drawing of the total embryo and determine the angle made by that section with the cephalo-caudal axis of the embryo. Draw in, at each point located on the median line, a cross line at the proper angle so determined. These lines represent the dorso-ventral axes of the transverse sections. Their lengths should correspond with those of the dorso-ventral axes of the drawings of the transverse sections previously made at the same magnification, $20\times$.

6. Plot in on each section-plane line (dorso-ventral axis) the dorsal and ventral boundaries of the neural tube as determined from measurements of the drawings already made. Interpolate by direct measurement and magnification of these points on intervening sections.

7. Sketch in the contours of the neural tube by connecting up the points which have just been plotted. Compare the drawing with a sagittal section of an embryo in the same stage of development.

Plastic reconstruction. — This method also will be indicated by practical directions for the reconstruction of a particular organ, in this case, a model $50\times$ of the heart of a 10 mm. pig, from a series of transverse section, 20μ in thickness.

1. Prepare a number of wax plates of the proper thickness. In this case, if every section is to be reconstructed, the thickness of the plates must be $50\times 20\mu$, or 1 mm. Nearly as good results can be obtained by reconstructing every second section and making the plates twice as thick. The wax is prepared according to the following formula:

Beeswax.....	6 parts
Paraffin, 56° C. m.p.....	4 parts
Rosin, white lump.....	2 parts
Mix and melt.	

Pour 130 grams of this wax into a pan with an inside measurement of 500×280 mm., into which boiling water has been poured to a depth of 15 mm. This amount of wax will make a plate 1 mm. in thickness. Bubbles in the wax may be removed by playing the flame of a bunsen burner over the surface as it is cooling. As the surface hardens, cut the edges free from the sides of

the pan. When the wax has set but is still malleable, roll up the plate and remove it to a soapstone slab, where it is unrolled and allowed to cool.

2. With the help of a camera lucida or projection apparatus, prepare outlines $50\times$ of the heart in all the sections in which it is found. Number the drawings consecutively and note the serial number of the sections drawn, so that it will be possible to check the drawings later if necessary. Note also whether the right and left sides of the drawing actually correspond with the right and left sides of the embryo or whether this condition is reversed. This is very important, as a mistake at this point would render the reconstruction valueless.

3. Transfer the drawings to the wax plates by means of carbon paper. Place the wax plates on a sheet of glass, and cut out the parts to be preserved with a sharp scalpel, leaving bridges of wax to connect the parts which would otherwise be separated. These bridges are best made in the form of arches bending towards the outside of the section.

4. Pile the sections in order, taking care to avoid the reversal of right and left sides, and to get an accurate fit. It is best to group the sections in piles of ten. A steady pressure of the hand will be sufficient to cause the sections to adhere to each other. The bridges may be cut away and stout pieces of wire substituted. Heat the wire at each end and press into position. After the wire is set, the wax bridges are cut away and the edges of the piece smoothed with a heated scalpel or aluminum modeling tool.

5. When all the sections have been combined in groups of ten, these groups should be united and the completed model smoothed in the same way. Such models may be painted or dissected, and mounted on wooden supports as desired. They are quite permanent if not exposed to high temperatures. Plaster of Paris molds and casts may be made from them in the customary manner.

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